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PHYSICAL ACTIVITY AND TYPE 1 DIABETES: IMPACT ON DIABETIC COMPLICATIONS

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ACADEMIC DISSERTATION

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"The greatest enemy of knowledge is not ignorance,
it is the illusion of knowledge."

Stephen Hawking

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I Johan Wadèn*, Heidi K. Tikkanen*, Forsblom C, Harjutsalo V, Thorn LM, Saraheimo M, Tolonen N, Rosengård-Bärlund M, Gordin D, Tikkanen HO, Groop P-H. Leisure-time physical activity and development of renal disease in type 1 diabetes; the FinnDiane Study. Diabetologia 58:929-936, 2015

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*Equal contribution

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ABBREVIATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes
AGE	Advanced glycation end-product
ARB	Angiotensin receptor blocker
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary artery disease
CAN	Cardiovascular autonomic neuropathy
CVD	Cardiovascular disease
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCCT	The Diabetes Control and Complications Trial
DN	Diabetic nephropathy
DM	Diabetes Mellitus
EDC	Pittsburgh Epidemiology of Diabetes Complications Study
EDIC	Epidemiology of Diabetes Interventions and Complications Study
ESRD	End-stage renal disease
FinnDiane	The Finnish Diabetic Nephropathy Study
GAD	Glutamate decarboxylase
GFR	Glomerular filtration rate
HbA_{1c}	Haemoglobin A1c
HLA	Human leukocyte antigen
HR	Hazard ratio
ICD	International Classification of Diseases
IQR	Interquartile range
LDL	Low-density lipoprotein
LTPA	Leisure-time physical activity
MDRD	Modification of Diet in Renal Disease
MET	Metabolic equivalent
MODY	Maturity onset diabetes of the young
OR	Odds ratio
PKC	Protein kinase C
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized controlled trial

ROS	Reactive oxygen species
SBP	Systolic blood pressure
SD	Standard deviation
TGF-β	Transforming growth factor-β
UAER	Urinary albumin excretion rate
UKPDS	UK Prospective Diabetes Study
VO_{2max}	Maximal oxygen uptake
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHO	World Health Organization
WHR	Waist-to-hip ratio

ABSTRACT

Background

Type 1 diabetes is a chronic condition with risk of severe long-term complications (cardiovascular disease, diabetic nephropathy, neuropathy and retinopathy) that increase the risk of premature mortality, reduce quality of life and cause a huge economic burden to society. The main cause of death and inability in individuals with type 1 diabetes are cardiovascular events, and it has been shown that diabetic nephropathy is the main driver of the increased risk of cardiovascular morbidity and mortality. Diabetic retinopathy is the leading cause of vision loss and blindness in developed countries. Physical activity has been shown to improve the risk profile of individuals with type 1 diabetes. Consequently, previous cross-sectional data show that lower physical activity is associated with a higher degree of diabetic complications, but the causal relationship is unclear.

Aim

The aim of this thesis is to assess how the total amount of leisure-time physical activity (LTPA) and its components of intensity, frequency and duration are associated with the development of diabetic nephropathy, cardiovascular outcomes, diabetic retinopathy and mortality in type 1 diabetes.

Subjects and methods

The study subjects of this thesis are participants in the ongoing nationwide, multicentre Finnish Diabetic Nephropathy (FinnDiane) Study. Currently, more than 5000 individuals with type 1 diabetes have been recruited and thoroughly characterized from all over Finland. LTPA was assessed at baseline by a validated self-report questionnaire. The study design is prospective and observational.

Results

The intensity of LTPA was associated with the initiation and progression of diabetic nephropathy. Of the other LTPA components, frequency was also associated with

the progression of diabetic nephropathy. In contrast, the total amount or duration of LTPA was not associated with the development or progression of diabetic nephropathy.

A larger amount of total LTPA and its components were associated with lower risk of incident CVD events. The association between LTPA frequency and incident CVD remained significant after adjustment for potential confounders. Also, LTPA intensity showed a borderline effect on the recurrence-free time of CVD events in individuals with a previous CVD event.

LTPA and all its components were associated with lower risk of all-cause mortality after adjusting for several confounders. However, only the LTPA intensity was associated with cardiovascular death after adjusting for covariates. Also, total LTPA and frequency of LTPA were independently associated with lower risk of mortality in individuals with type 1 diabetes and chronic kidney disease.

Frequent LTPA was associated with lower risk of severe diabetic retinopathy. The total amount or other components of LTPA (intensity or duration of a single session) were not associated with severe diabetic retinopathy, however.

Conclusions

Physical activity was associated with reduced risk of diabetic complications and mortality in individuals with type 1 diabetes. In addition, physical activity also seems to benefit those with diabetic complications – notably, diabetic nephropathy – and appears to be safe.

TIIVISTELMÄ

Taustaa

Tyypin 1 diabetes on krooninen sairaus, johon liittyy vakavia komplikaatioita (sydän- ja verisuonisairaus, munuaistauti eli nefropatia, hermostosairaus eli neuropatia ja silmän verkkokalvosairaus eli retinopatia). Nämä liitännäissairaudet lisäävät ennenaikaisen kuoleman vaaraa, heikentävät elämänlaatua ja lisäävät huomattavasti terveydenhuollon kustannuksia. Sydän- ja verisuonisairaudet ovat tärkein kuolleisuutta ja sairastavuutta lisäävä komplikaatio tyypin 1 diabeteksessa. On osoitettu, että diabeettinen nefropatia on tärkein tätä riskiä lisäävä tekijä. Diabeettinen retinopatia on keskeinen syy näön heikentymiselle ja sokeudelle kehittyneissä maissa. Fyysisen aktiviteetin on osoitettu parantavan tyypin 1 diabetesta sairastavien potilaiden riskijakaumaa. Aiemmat poikkileikkaustutkimukset ovat osoittaneet, että vähäinen liikunta liittyy diabeteksen komplikaatioiden lisääntymiseen, mutta syy-seuraussuhde on epäselvä.

Tavoitteet

Tämän väitöskirjan tavoitteena on arvioida, miten vapaa-ajan liikunta (kokonaismäärä, suorituksen kesto, intensiteetti ja frekvenssi) vaikuttaa komplikaatioiden kehittymiseen ja kuolleisuuteen tyypin 1 diabeteksessa.

Tutkimusaineisto ja menetelmät

Väitöskirjan potilaat ovat osana jatkuvaa, koko Suomen kattavaa, FinnDiane–monikeskustutkimusta. FinnDiane tutkimukseen on osallistunut jo yli 5000 suomalaista tyypin 1 diabetesta sairastavaa potilasta. Tutkimuksessa potilaiden fyysinen aktiivisuus arvioitiin suomalaisiin olosuhteisiin validoidulla liikuntakyselylomakkeella. Tutkimus on luonteeltaan havainnoiva seurantatutkimus.

Tulokset

Vapaa-ajan liikunnan vähäinen intensiteetti liittyi diabeettisen munuaissairauden aikaiseen ilmaantumiseen ja nopeaan etenemiseen. Lisäksi liikuntakertojen

määrä oli vastaavasti yhteydessä diabeettisen nefropatian ilmaantumiseen ja etenemiseen. Suurempi fyysisen aktiivisuuden määrä liittyi matalampaan sydän- ja verisuonikomplikaatioriskiin. Tuloksen tilastollinen merkitsevyys säilyi, vaikka mahdolliset sekoittavat tekijät huomioitiin. Fyysisen aktiivisuuden määrä oli yhteydessä kokonaiskuolleisuuteen myös munuaissairautta sairastavilla potilailla. Suurempi liikuntakertojen määrä liittyi pienempään riskiin sairastua diabeettiseen retinopatiaan.

Johtopäätökset

Fyysinen aktiivisuus on yhteydessä vähäisempään riskiin sairastua diabeteksen komplikaatioihin ja kuolla ennenaikaisesti. Fyysinen aktiivisuus on hyödyllistä myös potilailla, joilla on jo liitännäissairauksia, kuten diabeettinen munuaissairaus.

1 INTRODUCTION

Diabetes, a common chronic disease characterized by elevated blood glucose, constitutes a major global health problem. In 2014, the World Health Organization (WHO) estimated that 422 million adults are affected by diabetes. In addition, almost half the individuals living with diabetes are estimated to be undiagnosed (1). The most common forms of diabetes are type 1 and type 2 diabetes. Type 2 diabetes is characterized by insulin resistance and relative insulin deficiency. Type 1 diabetes, on the other hand, is characterised by a total loss of endogenous insulin production, and if left untreated, it leads to ketoacidosis and premature death. Therefore, until the discovery and availability of exogenous insulin in 1922, type 1 diabetes was a fatal disease with no cure.

For reasons unknown, the incidence of type I diabetes is increasing worldwide in both low- and high-incidence populations (2). The onset of diabetes is shifting towards younger patients, although the incidence has increased in all age groups (3). Finland has the highest age-standardized incidence rate of type 1 diabetes in the world, with more than 60 per 100 000 per year in individuals below the age of 15 (4). Of the estimated half a million people affected by diabetes in Finland, a total of 50 000 have type 1 diabetes (5).

Although the quality of diabetes care and modes of insulin treatment have improved significantly over the years, type 1 diabetes is still associated with severe long-term complications that increase the risk of premature death and disability. These complications include microvascular complications such as diabetic nephropathy, diabetic retinopathy and diabetic neuropathy, as well as cardiovascular complications such as coronary artery disease, myocardial infarction, stroke and peripheral vascular disease. Cardiovascular disease (CVD) is the most common cause of death and disability among individuals with type 1 diabetes. Diabetic nephropathy (DN) accounts for a large extent of the increase in cardiovascular morbidity and mortality in these patients (6). It is of note that an elevated concentration of glucose in the circulation is needed (a prerequisite) for the development of these complications, although there are many other modifiable risk factors involved. Therefore, the treatment strategy for type 1 diabetes includes a multifactorial approach targeting all modifiable risk factors and not only optimizing the glycaemic control.

Physical activity has been shown to provide multiple significant health benefits in the general population and in individuals with type 2 diabetes (7). Importantly, physical activity has been instrumental in the primary and secondary prevention of type 2 diabetes and has been shown to reduce the risk of CVD and mortality in exposed individuals (7-10). In addition, it has been shown to improve several

vascular risk factors implicated in the pathogenesis of diabetic complications. For example, physical activity improves glucose and lipid control and insulin resistance and reduces body weight and blood pressure (7).

However, there are surprisingly limited prospective data evaluating the causal effects of physical activity on the development of diabetic complications in type 1 diabetes. No previous prospective studies have assessed the association of physical activity and the progression of diabetic nephropathy. In fact, physical activity guidelines for individuals with type 1 diabetes rely largely on information obtained from studies in type 2 diabetes or the general population (11). It is obvious that there is an urgent demand for evidence regarding which type of physical activity is most beneficial in terms of intensity, duration, volume and type for individuals with type 1 diabetes. Therefore, in this thesis, the aim is to evaluate how leisure-time physical activity and its components (intensity, frequency, volume and duration) are associated with the development of diabetic complications and premature mortality in individuals with type 1 diabetes.

2 REVIEW OF THE LITERATURE

2.1 Diabetes Mellitus

2.1.1 Definition

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronically elevated blood glucose concentration due to defects in insulin secretion, insulin action or a combination of these two, leading to disturbed carbohydrate, fat and protein metabolism. The condition is associated with both serious acute and long-term complications affecting the quality of life and the life expectancy of the individual with DM. (12)

2.1.2 Classification of diabetes

Based on their pathogenesis, the majority of cases with DM can be divided into two major classes, namely type 1 and type 2 diabetes (12). Type 1 diabetes, previously referred to as insulin-dependent diabetes or juvenile-onset diabetes, is characterized by autoimmune-mediated B-cell destruction in the pancreatic islets, leading to a loss of insulin secretion (12, 13). Type 2 diabetes, previously referred to as non-insulin-dependent diabetes, is often linked to obesity with subsequent resistance to the insulin action in the target tissues and/or lack of insulin secretion as the disease progresses. However, because of disease heterogeneity and substantial overlap between the two major DM conditions, labelling a particular diabetes type is sometimes challenging (14).

There are also other forms of diabetes. Gestational diabetes is characterised by insulin resistance and hyperglycaemia discovered during pregnancy. This hyperglycaemia usually improves or disappears after labour. However, women with gestational diabetes have an increased risk of developing type 2 diabetes during follow-up. (15) While type 1 and type 2 diabetes are complex genetic disorders (caused by many genes), there are also rare monogenetic forms of diabetes, such as the various types of MODY (Maturity Onset Diabetes of the Young), of which MODY 3 is most common in Finland (16). There are also other rare monogenic conditions that cause diabetes. Notably, diabetes may also occur due to loss or damage of the pancreatic tissue caused by surgery, cancer, trauma or pancreatitis. Moreover, some drugs, e.g. glucocorticoids, have been shown to influence the glucose metabolism (17). Recently, a proposal for a new classification of diabetes based on the individual patient characteristics at diagnosis was introduced (18). These

characteristics include age at diagnosis, BMI, HbA_{1c}, glutamate decarboxylase (GAD) antibodies and the homeostatic model assessment (HOMA) 2 estimates of β-cell function and insulin resistance. Time will tell whether this novel classification will gain broader implementation.

2.1.3 Diagnostic criteria

A diabetes diagnosis is based on measurements of glycated haemoglobin, fasting plasma glucose or plasma glucose after an oral glucose tolerance test. If an individual presents with the classic symptoms of diabetes (weight loss, polyuria, polydipsia and/or polyphagia) or a hyperglycaemic crisis, the diagnosis can be made based on a single plasma glucose measurement. However, the diagnosis must be confirmed with a repeated diagnostic measurement on a different day for an asymptomatic individual. The WHO's (World Health Organization) diagnostic criteria for diabetes are a fasting plasma glucose level of ≥7.0 mmol/l (126 mg/dl), plasma glucose ≥11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load, symptoms of hyperglycaemia, and a random plasma glucose concentration of ≥11.1 mmol/l (200 mg/dl) (19). The ADA (American Diabetes Association) has included a glycated haemoglobin of 48 mmol/mol (6.5%) or higher in their guidelines for a diagnosis of diabetes (14).

2.1.4 Type 1 Diabetes – Epidemiology and Pathogenesis

Type 1 diabetes accounts for 5–10% of diabetes cases. The incidence of type 1 diabetes is rapidly growing worldwide, indicating that lifestyle/environmental factors in genetically susceptible individuals play an important role for the onset of the disease (3). The geographical variation in the incidence of type 1 diabetes is large, with incidence rates (in children under 15 years) varying from 0.1 per 100 000/year in parts of Venezuela and China to 22 per 100 000/year in Canada to over 60 per 100 000/year in Finland, which has the highest incidence rate of type 1 diabetes in the world (3, 20, 21). The age at onset of type 1 diabetes has been shifting towards the younger age groups, and there is a slight male excess in the individuals diagnosed with type 1 diabetes, whereas other autoimmune diseases are more common in women (3). Although the incidence of type 1 diabetes is highest in children, it is of note that the diagnosis can be made at any age.

Type 1 diabetes is an autoimmune disease characterised by gradual immune-mediated B-cell destruction of the pancreatic islets of Langerhans, resulting in a life-long dependence on exogenous insulin. Activation of the immune system by environmental triggers leads to an inflammatory response in susceptible individuals. A chain of functional defects in the bone marrow and thymus, immune system, and β cells together contribute to the pathogenesis of type 1 diabetes. (13) Symptoms of

diabetes occur when 80–90% of the β cells have been destroyed due to a chronic inflammatory process affecting the islets (22, 23). Islet autoantibodies, e.g. insulin autoantibodies (IAA), GAD antibodies, islet cell antibodies (ICA), insulinoma-associated antigen-2 (IA-2A) and zinc transporter 8 antibodies (ZnT8) are detectable in 90% of individuals recently diagnosed with type 1 diabetes (24, 25). These antibodies can be detected years or even decades before the clinical manifestation of the disease by sensitive radioimmunoassays. The number of autoantibodies detected increases the risk of type 1 diabetes cumulatively (26). The presence of two or more autoantibodies – referred to as the point of no return – increases the risk of developing type 1 diabetes during the next 10 years to 70% (27).

So far, over 50 genetic loci have been found that affect the genetic predisposition to type 1 diabetes. The majority of the identified gene loci are thought to involve immune responses. (28) The human leukocyte antigen (HLA) locus accounts for half of the genetic susceptibility to the disease (29). Of the many HLA types, the HLA class II shows the strongest association with type 1 diabetes. Also, genes that protect against type 1 diabetes have been identified (30). Many of these genetic markers are common in the Finnish population. However, only a relatively small proportion of individuals with risk alleles finally develop clinical disease (31).

It is obvious that the rapidly increasing incidence rate of type 1 diabetes cannot be explained by changes in the genetic pool. Interestingly, immigrants with different ethnic backgrounds from low-incidence risk regions seem to be at higher incidence risk among the population in the new area (32). Further, the incidence rise in childhood disease manifestation is associated with weaker contributions from high-risk HLA haplotypes (33). Therefore, an environmental/lifestyle influence is clear yet poorly understood. Various environmental factors including certain viruses, toxins and dietary factors may contribute, and hypotheses such as the "accelerator hypothesis" and the "hygiene hypothesis" have been suggested to explain the rapid increase in the type 1 diabetes incidence rate (34–36).

2.1.5 Type 1 Diabetes – Treatment

Before the milestone in the history of diabetes – the discovery of exogenous insulin in 1921 – diabetes was a fatal disease (37). Animal-derived insulin was the first insulin to be administered to humans, followed by human insulin and, further, by insulin analogues. After being diagnosed with type 1 diabetes, a patient can require minimal exogenous insulin for a time due to a partial recovery of β-cell function, a phenomenon called the "honeymoon period". Interestingly, 30–80% of individuals with type 1 diabetes preserve small amounts of residual endogenous insulin production assessed by c-peptide measurement (38). This has been shown to be associated with less retinopathy and less hypoglycaemia during follow-up, making it an intriguing therapeutic target in the future (39).

Insulin treatment can be given in different ways in order to achieve the general objective of lowering blood glucose to the near normal range of HbA_{1c}, less than 53 mmol/mol (or less than 7.0%). However, the blood glucose targets are always individual, and less or more stringent HbA_{1c} goals are sometimes appropriate. (40) Insulin can be administered through multiple daily injections or continuous subcutaneous insulin infusion (CSII) therapy. With multiple daily injections (i.e. basal-bolus therapy), long-acting basal insulin is injected once or twice a day, providing the basal insulin supplementation, and rapid-acting bolus insulin is given before meals based on carbohydrate intake. The aim is to mimic physiologic insulin and glucose profiles. However, studies have shown that a greater HbA_{1c} reduction can be achieved with continuous subcutaneous insulin infusions (41). In insulin pump therapy, there is a constant infusion of rapid-acting insulin, which can vary hour by hour to meet the individualised physiological needs. This is accompanied by additional bolus insulin administered by the patient before meals, as in the multiple daily injection therapy. Continuous glucose monitoring has resulted in a significant reduction in time spent in hypoglycaemia paralleled by a reduction in HbA_{1c} compared to the self-monitoring of blood glucose (42). The most recent technological advancement in diabetes care is a hybrid closed loop system that integrates the insulin pump to deliver insulin and/or glucagon with continuous glucose monitoring systems, i.e. the artificial pancreas, which was approved by the FDA in 2016 (43). Studies regarding closed loop systems have shown promising results regarding the glycaemic control and the risk of hypoglycaemic events (44).

Despite recent advancements, disease prevention, delay or cure, and the challenge to overcome the autoimmune nature of the disease, have proven surprisingly difficult. Although type 1 diabetes is a predictable disease, results from primary and secondary disease prevention studies have shown limited benefit so far (45, 46). For now, the goal of type 1 diabetes management is to achieve as close to normal blood glucose levels as possible without severe hypoglycaemia. The ultimate treatment goal, of course, is to prevent diabetic complications and improve the quality of life of the affected individuals. That goal requires a multidimensional approach, including optimal insulin treatment and management of risk factors, as well as a focus on healthy lifestyle. However, hopefully one day, the type 1 diabetes itself can be prevented or even cured.

2.2 Complications in type 1 diabetes

Type 1 diabetes is a chronic condition involving long-term disease-associated complications leading to excess morbidity and mortality and is a huge cost burden to society (47–49). Generally, these complications are classified as micro- or macrovascular: damage to the small blood vessels leads to microvascular

complications (diabetic nephropathy, retinopathy and neuropathy). Macrovascular disease, including coronary artery disease, cerebrovascular disease and peripheral arterial disease, is the most common cause of premature death and disability among people affected by type 1 diabetes (50).

One of the mainstays in the field of diabetic complications, Professor Michael Brownlee, proposed his unifying hypothesis for the development of both micro- and macrovascular complications in 2005. His theory suggested that hyperglycaemia induces oxidative stress, which is the unifying upstream event that activates the major metabolic pathways involved in diabetic tissue damage. Thus, mitochondrial overproduction of reactive oxygen species (ROS) causes damage via four major mechanisms: the polyol pathway, the hexosamine pathway, the production of advanced glycation end products (AGEs), and the activation of protein kinase C (PKC). Insulin resistance may further accelerate the glucose-induced tissue damage in macrovascular disease. (51)

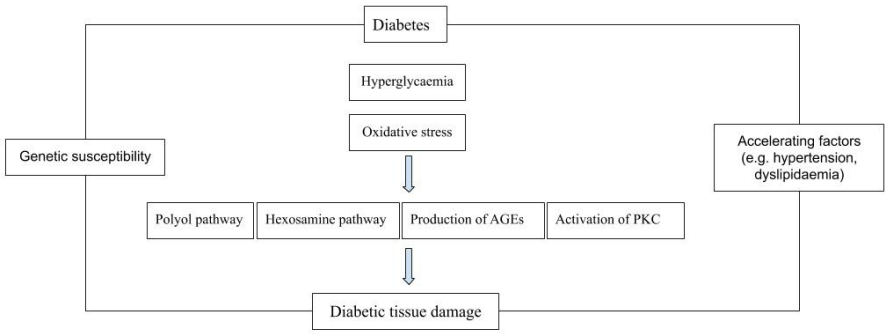


Figure 1. The Brownlee hypothesis. Modified from Brownlee et al., "The Pathobiology of Diabetic Complications", *Diabetes* (2005) (51).

2.2.1 Diabetic nephropathy

2.2.1.1 Definition, diagnosis and renal function

Diabetic nephropathy is the most common cause of chronic kidney disease (CKD) worldwide and accounts for the increased risk of premature mortality associated with type 1 diabetes (6). The natural history of diabetic nephropathy commences from subclinical disease characterised by renal hyperfiltration followed by the occurrence of persistent microalbuminuria. Later, the disease is characterised by a gradual decline of the GFR and increased leakage of albumin into the urine, eventually leading to the final stage of end-stage renal disease (ESRD).

Screening for microalbuminuria is the cornerstone of finding patients at risk for diabetic nephropathy. The urinary albumin excretion (UAER) is measured either

from a 24-hour or a timed overnight urine collection, and the patient is diagnosed if they have two positive samples out of three consecutive diagnostic measurements. The earliest sign of diabetic nephropathy is microalbuminuria (≥20 μg/min or ≥30 mg/24 h), and its presence is a strong predictor of progression to macroalbuminuria or overt nephropathy (defined as an UAER of ≥200 μg/min or ≥300 mg/24 h) and eventually to ESRD, defined as the demand for dialysis or renal transplant. (52, 53) The 24-hour urine collection has been regarded as the gold standard, although a more convenient and commonly used method to detect microalbuminuria is the albumin (μg)/creatinine (mg) ratio measured in a random urine sample. Earlier studies demonstrated microalbuminuria as a stride towards the later sequential stages of diabetic nephropathy, but more recent studies suggest that, in a substantial proportion of the individuals with type 1 diabetes, microalbuminuria can indeed regress (54). Some individuals may even follow a nonalbuminuric pathway to diabetic nephropathy, and in such cases, the GFR decline may have started even before the onset of albuminuria (55). Therefore, a new model was recently suggested in which the clinical feature of diabetic nephropathy in type 1 diabetes is progressive renal decline and not albuminuria (56). In fact, in type 2 diabetes, this phenomenon is common: as much as 30–57% of patients with chronic kidney disease (CKD) are normoalbuminuric (57–59). On the other hand, nonalbuminuric CKD is uncommon in type 1 diabetes and was found in only 2% of individuals with type 1 diabetes in the FinnDiane Study (60). In addition, normoalbuminuric CKD in this cohort was seen in elderly females and was predictive of cardiovascular disease rather than kidney disease. Nevertheless, microalbuminuria plays an essential diagnostic role in the assessment of early diabetic nephropathy and is the best non-invasive predictor of progression. In addition, microalbuminuria independently predicts cardiovascular morbidity and mortality (6, 61, 62).

A decrease in the glomerular filtration rate (GFR) to <60 mL/min/1.73 m^2 for three months or more is commonly used as a diagnostic criterion for CKD. Indirect measurement of GFR by inulin clearance during continuous inulin infusion is considered the gold-standard method for measuring renal function (63). However, this method is not feasible in the general clinical practice. Therefore, other markers and methods are being used to estimate renal function in both clinical practice and research. The most widely used methods are estimates based on the serum creatinine level: the Cockcroft-Gault formula (64), the Modification of Diet in Renal Disease (MDRD) (65), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (66). At the time, the most recent CKD-EPI equation is the most accurate GFR estimating equation that has been evaluated in large diverse populations and has, therefore, been proposed to replace the MDRD equation in clinical practice (67). Renal function is divided into five categories based on the estimation of the GFR (eGFR) (Table 1).

Table 1. The staging of CKD and GFR categories (National Kidney Foundation)

Stage	GFR ml/min/1.73 m2	Description
1	≥90	Normal or high
2	60–89	Mildly reduced
3a	45–59	Mildly to moderately reduced
3b	30–44	Moderately to severely decreased
4	15–29	Severely decreased
5	<15	Renal failure

2.2.1.2 Epidemiology

The presence of DN is associated with an increased risk of CVD, and it is the leading cause of renal failure and increased mortality among patients with type 1 diabetes in the developed world (68). About one-third of the individuals with type 1 diabetes develop DN. At first, the incidence increases linearly with the duration of diabetes, but 20–25 years after diagnosis of diabetes, the incidence begins to decline. However, the overall incidence of DN and ESRD has decreased, especially in individuals most recently diagnosed with type 1 diabetes, a phenomenon probably due to improved care. (54, 69, 70). In Finland, the incidence of ESRD has also decreased, and the cumulative incidence for the development of ESRD within 30 years is approximately 7.8% (71). It should be noted that, since the earliest studies in the 1980s, there is now a delay of 10 or more years in the appearance of ESRD, although there are differences between regions and even among the European countries (72). Previously, approximately 80% of the individuals with microalbuminuria developed overt proteinuria (52, 53), whereas our own data a few years later showed a much lower progression rate of 28% in individuals with a duration of diabetes over 15 years (73). As described earlier, even more than a third of individuals with microalbuminuria may regress to a normal albumin excretion rate, emphasizing the importance of early disease detection and intervention (74, 75).

2.2.1.3 Pathogenesis

Genetic predisposition, accompanied by environmental factors and the diabetic milieu, leads to structural and functional abnormalities in DN. Functional and haemodynamic abnormalities include early glomerular hyperfiltration, glomerular hypertension (i.e. increased intraglomerular pressure), increased permeability of the basement membrane, shear stress and increased albumin excretion. As nephropathy progresses, the proteinuria increases and the GFR deteriorates. (76) In addition, hyperglycaemia induces the production of humoral mediators, cytokines and growth factors, resulting in structural changes such as glomerular mesangial expansion

and thickening of the glomerular basement membrane. As the disease progresses, mesangial expansion typically presents as glomerulosclerosis, with characteristic nodular lesions called the Kimmelstiel-Wilson nodules. Tubulointerstitial injury develops alongside the glomerular damage. In the early stages of diabetic nephropathy, tubular hypertrophy is present, but thereafter, interstitial fibrosis with tubular atrophy develops, accompanied by arteriolar hyalinosis. (77,78)

Several pathophysiological pathways are involved in the development of diabetic nephropathy. A hormonal system that regulates blood pressure and fluid homeostasis, i.e. the renin-angiotensin-aldosterone system (RAAS) is an essential element in the pathogenesis of DN (78,79). Hyperglycaemia induces the production of angiotensin II, the main peptide of the RAAS, as well as extracellular matrix accumulation by mesangial cells, primarily via stimulation of transforming growth factor-β (TGF-β) expression. TGF-β has been viewed as a key player in mediating the profibrotic and hypertrophic effects of various pathological stimuli. (80, 81) In addition, angiotensin II constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressures and direct podocyte injury through the generation of ROS and an increased influx of calcium. Notably, there is inappropriate overproduction of angiotensin II predisposing to hypertension, a condition that often accompanies diabetes and accelerates the progression of diabetic nephropathy. (77-79) Moreover, renal hyperfiltration is an early haemodynamic abnormality associated with increased glomerular pressure and potentially the development of diabetic nephropathy. The most recent oral glucose-lowering agents, sodium-glucose cotransporter 2 inhibitors, have shown renal protection by normalising hyperfiltration via tubuloglomerular feedback. (82) There are several other known complex pathways overlapping and interacting with one another and thereby contributing to the development of DN. Hyperglycaemia, the main driver of the development of DN, results in the up-regulation of the glucose transporter GLUT-1, further induced by increased intraglomerular pressure, Ang-II and an abnormal stimulation of renal cells to produce more TGF-β1, thereby amplifying the loop of glucotoxicity. Hyperglycaemia induces vascular injury through the generation of superoxide, hydroxyl radical, hydrogen peroxide and peroxynitrite, all commonly referred to as ROS. (83,84) Oxidative stress has been suggested as the unifying mechanism in the pathogenesis of DN via different metabolic pathways: the polyol pathway, hexosamine pathway, the production of AGEs and activation of PKC, as pointed out above (84–86). Other factors that contribute to the pathogenesis of DN include the elaboration of proinflammatory cytokines and activation of growth factors such as connective tissue growth factor, insulin-like growth factor-1 and vascular endothelial growth factor (87, 88).

2.2.1.4 Risk factors

Several risk factors have been shown to contribute to the development of DN in individuals with type 1 diabetes. Risk factors such as hyperglycaemia, high blood pressure, dyslipidaemia, insulin resistance and smoking are supported by a poorly characterised genetic background. However, the genetic factors are likely to increase susceptibility to the complication. For example, familial aggregation of DN is shown in studies regarding both type 1 and type 2 diabetes, and a parental history of hypertension or CVD predicts its development (89–93). There are also differences in the incidence of DN between groups of different ethnic backgrounds (94, 95). Furthermore, in the Wisconsin Epidemiologic Study, Klein et al. showed that a large proportion of individuals with type 1 diabetes did not develop DN despite severe and chronic hyperglycaemia (96). Some of the risk factors will be discussed in detail below.

Glucose

An elevated blood glucose concentration is a prerequisite for the development of DN. Several studies have shown that, by improving the glycaemic control in individuals with type 1 diabetes, their risk of diabetic nephropathy during follow-up is substantially reduced. The landmark Diabetes Control and Complications Trial (DCCT) established the importance of glycaemic control and confirmed the previous findings from the Steno, Oslo and Stockholm studies, which showed renal benefits through the lowering of HbA1c (97–100). These data resulted in a global change in the management of type 1 diabetes. The DCCT randomised 1441 individuals with type 1 diabetes to receive either intensive or conventional glucose-lowering therapy and confirmed the importance of optimal blood glucose control in order to reduce the risk of complications in type 1 diabetes. Over 6.5 years of follow-up, intensive glycaemic control (median HbA1c, 7.2% vs. 9.1% in the conventionally treated group) reduced the cumulative incidence and overall risk of microalbuminuria by 39% and macroalbuminuria by 54% in both the primary prevention and secondary intervention groups. Observational follow-up data on the same patients after the close-out of the DCCT showed that, although the HbA1c levels were similar during the EDIC (Epidemiology of Diabetes Interventions and Complications) study follow-up, the risk of DN in the former intensively treated group was significantly lower than in the conventionally treated group. This indicated that exposure to hyperglycaemia during DCCT was remembered in the "metabolic memory", and based on this observation, it is important to establish stringent glycaemic control early in the course of type 1 diabetes (101). On the other hand, the most significant side effect of tight glycaemic control was severe hypoglycaemia, which highlights the importance of individualised treatment. It is of note that patients with the same HbA_{1c} levels might have markedly different

glucose profiles with frequent hypo- and hyperglycaemic values, and thus, glucose variability may be an additional risk factor for the development of complications, particularly diabetic nephropathy. Interestingly, a retrospective analysis of the data from the DCCT led to the consideration that glycaemic variability may influence the development of complications in type 1 diabetes apart from the mean HbA_{1c} (102). More recent data supported the initial finding that glycaemic variability may be important in the development of diabetic nephropathy in type 1 diabetes, but larger prospective outcome studies are required to verify these preliminary findings (103, 104). In addition, recent data from the DCCT showed that the time in range of 70–180 mg/dL (3.9–10 mmol/L) was strongly associated with reduced microvacular complications (105).

Hypertension

Hypertension is common in individuals with type 1 diabetes, even in those without renal involvement, but it is definitely more common in those individuals, who progress to microalbuminuria (106, 107). Blood pressure contributes considerably to the development and progression of diabetic nephropathy and is associated with both the increase of proteinuria and the decrease of renal function (108, 109). The prognosis of DN has improved in recent decades – largely because hypertension is being more aggressively treated (110). A recent meta-analysis suggested that angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARB) were the most effective strategies against ESRD (111). Also, current international blood pressure management guidelines suggest that these agents are similarly effective in the prevention of renal failure in individuals with CKD, but it should be noted that combining them is not recommended due to an excess of renal adverse events (112). RAAS inhibition is renoprotective, even in normotensive type 1 diabetes individuals with microalbuminuria. Interestingly, the Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) showed that RAAS inhibition prevented the onset of microalbuminuria in type 2 diabetes (113). However, whether microalbuminuria could be prevented by RAAS inhibition in type 1 diabetes is still unknown. So far, studies have not shown this benefit in type 1 diabetes (114).

Insulin resistance

Insulin resistance, which is related to hypertension, dyslipidaemia and cardiovascular events, has also been proposed to play a central role in the development and progression of diabetic nephropathy. There are several studies supporting this proposal. The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study showed that insulin sensitivity measured by the estimated glucose disposal rate predicted DN (115). In addition, it has been shown that insulin sensitivity is associated with microalbuminuria by using the euglycaemic hyperinsulinaemic clamp technique, the gold standard in measuring insulin sensitivity (116–118).

Obesity

Obesity is highly prevalent and is a growing problem in individuals with type 1 diabetes (119). In fact, several studies have shown an association between the metabolic syndrome (visceral obesity, dyslipidaemia, hyperglycaemia and hypertension) and the risk of DN in type 1 diabetes (120, 121). In the general population, the relative risk of ESRD increases progressively with the degree of overweight/obesity, and some studies also show an association between obesity and DN in type 1 diabetes (122–125). These data were recently replicated and extended to show a causal relationship between obesity and diabetic nephropathy by our own research group (126).

Other risk factors

Several other risk factors contribute to the development of DN in type 1 diabetes. Data from the FinnDiane Study show that dyslipidaemia predicted the initiation and progression of diabetic nephropathy at all stages of renal disease in type 1 diabetes (127–129). In line with these findings, dyslipidaemia has been shown to associate with renal disease in the DCCT/EDIC, EURODIAB and Estudio Diamante cohorts (130–132). Other risk factors such as smoking (133), inflammatory markers (134, 135), male gender (136), low birth weight (137) and short adult stature are also associated with the development of diabetic nephropathy in type 1 diabetes (138, 139).

2.2.2 Diabetic Retinopathy

Diabetic nephropathy and diabetic retinopathy are closely associated. Diabetic retinopathy results from damage to the small blood vessels and neurons in the retina and is a leading cause of blindness in developed countries (140). It was estimated that, in the USA, 86% of the individuals with type 1 diabetes have diabetic retinopathy, and about one-half have vision-threatening retinopathy (141). Likewise, high prevalence estimates have been reported in other countries (142). Eventually, as the duration of their diabetes gets longer, all individuals with type 1 diabetes will develop some degree of retinopathy (143). However, there has been a significant reduction in the incidence, progression and prevalence of diabetic retinopathy over the past several decades in individuals with a more recent diagnosis of type 1 diabetes due to improved treatment of dyslipidaemia, hypertension and hyperglycaemia (144, 145). Consequently, in the WESDR study, the estimated annual incidence of proliferative diabetic retinopathy decreased by 77% and vision impairment by 57% between 1980 and 2007 (146, 147).

Along with the risk of vision loss, diabetic retinopathy is accompanied by a higher risk of mortality and systemic vascular complications, including stroke, coronary heart disease, heart failure and DN (148). The most firmly established risk factors

for diabetic retinopathy are diabetes duration (149) and hyperglycaemia (150). Hypertension is an important modifiable risk factor (151), and diabetic retinopathy is further associated with risk factors such as obesity (152), dyslipidaemia (153), pregnancy (154) and puberty (155, 156).

The asymptomatic first stage of diabetic retinopathy, called non-proliferative diabetic retinopathy, is characterised by the presence of early intraretinal microvascular findings of microaneurysms, haemorrhages, retinal oedema, lipid exudates and microinfarcts (157). These are found in almost all individuals with type 1 diabetes after 20 years of diabetes (143). Treatment of blood pressure, optimal serum lipid and glycaemic control can delay the onset and slow the progression of non-proliferative diabetic retinopathy to the later-stage sight-threatening neovascularisation of the retina designated as proliferative retinopathy.

Macular oedema can occur at any stage of diabetic retinopathy and can lead to blindness. It is characterised by macular swelling caused by the leakage of fluids and lipids into the macula from damaged blood vessels (158). Diabetic retinopathy is diagnosed with fundus photography or direct ophthalmoscopy. For adults, international guidelines advocate for diabetic retinopathy screenings five years after the diabetes diagnosis (159).

The DCCT, the UK Prospective Diabetes Study (UKPDS), and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Eye Study have shown that intensive glycaemic control reduces the risk of development and progression of diabetic retinopathy (150, 151, 160). In the DCCT trial, even though the mean HbA_{1c} levelled off during the observational follow-up, the benefit of the intensive treatment persisted (161). The UKPDS study showed that lowering the mean systolic blood pressure (SBP) from 154 mmHg to 144 mmHg slowed the progression of diabetic retinopathy in individuals with type 2 diabetes (160). Findings from observational studies further suggest that dyslipidaemia is associated with the progression of diabetic retinopathy (158). Thus, the comprehensive treatment of all risk factors for diabetic retinopathy constitutes the foundation of the prevention and management of the complication (151). Finally, interventions such as laser photocoagulation, vitrectomy and injections of anti-vascular endothelial growth factor are effective for the prevention of visual impairment in more advanced stages of diabetic retinopathy (severe non-proliferative retinopathy, proliferative retinopathy and macular oedema) (158).

2.2.3 Diabetic neuropathy

Diabetic neuropathy refers to nerve damage associated with diabetes and confers the increased risk of other diabetic complications and premature mortality (162). The estimated prevalence for diabetic neuropathy ranges widely based on the study population and definition of diabetic neuropathy. Microvascular injury to

the blood vessels supplying nerves and nerve damage results in diverse clinical manifestations. Risk factors for diabetic neuropathy include hyperglycaemia, long diabetes duration, diabetic retinopathy, microalbuminuria, obesity, smoking, dyslipidaemia, hypertension and tall stature (163–167).

Different classifications of diabetic neuropathy have been proposed throughout the history of diabetes. Currently, the most common way to classify diabetic neuropathies is to divide them into two major forms: generalised and focal neuropathy. The most common form is chronic sensorimotor distal symmetric polyneuropathy (DPN), which is diagnosed by clinical tests such as vibration perception, 10-g monofilament perception, temperature perception and ankle reflex testing. Individuals with five years or more of type 1 diabetes should be assessed annually. Early detection of DPN together with peripheral arterial disease is important to prevent foot ulcers and, consequently, limb amputation since a major proportion of patients with DPN may be asymptomatic. (168) About 40–60% of individuals that have documented neuropathy suffer from neuropathic pain (169). However, this might be an underestimation since patients often do not mention these symptoms to their attending physicians (170).

Among the neuropathies, cardiac autonomic neuropathy (CAN) is the most studied and the most clinically significant diabetic autonomic neuropathy. The prevalence of CAN increases with the duration of diabetes, and prevalence rates of at least 30% were observed in the DCCT/EDIC cohort after 20 years of diabetes onset (171–173). Several longitudinal studies have shown that CAN is associated with significant increases in morbidity and mortality (174-178). Consequently, CAN is an independent risk factor for cardiovascular mortality, arrhythmias, silent ischaemia, major cardiovascular events and myocardial dysfunction. (174–179) Importantly, CAN may cause exercise intolerance and may also increase the risk of exercise-related sudden death (180, 181). Hyperglycaemia is the major risk factor for CAN (182, 183), and the DCCT/EPIC trials showed that glucose control with a near-normal glycaemia target substantially reduces the incidence of CAN in individuals with type 1 diabetes (184). Other risk factors associated with CAN are age, elevated blood pressure and presence of other diabetic complications (185–188). Typically, the early stages of CAN are asymptomatic, and the earliest finding is a decrease in heart rate variability, which can be detected by a deep breathing test. Later, resting tachycardia, symptoms of orthostatic hypotension and abnormal blood pressure profile, arrhythmias and exercise intolerance may appear. Other neuropathies include typical focal neuropathies such as carpal tunnel syndrome, diabetic amyotrophy and nerve palsies. (189)

2.2.4 Macrovascular complications

Macrovascular complications of diabetes include coronary artery disease (CAD), cerebrovascular disease and peripheral arterial disease (PAD). Atherosclerosis is at the core of the pathogenesis of these complications. In the majority of studies regarding CVD and type 1 diabetes, the outcomes are pooled together in a common CVD endpoint. CAD is the most common and studied macrovascular complication of diabetes, and there are fewer data on cerebrovascular disease and peripheral arterial disease in type 1 diabetes. Individuals with type 1 diabetes are at substantially increased risk of premature mortality, and cardiovascular disease (CVD) is the most important cause of death in these individuals (50). Altogether, CVD is more common and occurs earlier in type 1 diabetes than in the general population. For example, in the UK's General Practice Research Database (GPRD) study, CVD events developed 10–15 years earlier than in the matched control subjects without diabetes (190). The risk of CVD in type 1 diabetes varies among study cohorts. Several studies show that the age-adjusted relative risk for CVD in type 1 diabetes is around 10 times higher than that of the general population (191–193).

The presence of diabetic nephropathy is strongly associated with CVD. In the Steno cohort, there was a 4.2-fold increase in the relative risk of CVD mortality in individuals with type 1 diabetes without proteinuria and a 37-fold increase in the risk of CVD mortality in those with proteinuria compared to the general population (194). Interestingly, in the FinnDiane Study, individuals with type 1 diabetes with normal UAER had a similar standardised mortality rate to the general population. However, individuals with microalbuminuria showed a 3-fold higher risk of all-cause mortality, and those with ESRD had an 18-fold higher risk during follow-up. (6) A similar finding was also observed in the Pittsburgh cohort, in which the presence of microalbuminuria or DN accounted for the increased cardiovascular mortality in type 1 diabetes (195). Notably, there is a decreasing trend regarding all-cause mortality and the incidence of cardiovascular complications in type 1 diabetes. Such decreasing incidence rates were recently shown in the Swedish nationwide registry data and were coherent with trends observed in other studies in North America and Europe (194, 196–200). However, there has been a more significant decline in the incidence of DN in type 1 diabetes in recent decades than in the CVD events, suggesting that these complications are not identical even though share risk factors (201). In the general population, female gender is known to protect from CVD. However, in type 1 diabetes, the protective effect seems to be eliminated. A number of studies have shown similar rates of CVD events in both genders (202–206), but one study showed that the relative mortality risk for women with CAD was 40-fold compared to the general population, whereas for men, it was 10-fold higher (207). Also, a family history of type 2 diabetes or CVD events is associated with CVD events in type 1 diabetes (208, 209).

Previous observational data suggest that dysglycaemia is a risk factor for CVD events in type 1 diabetes, although the published studies have shown some inconsistencies. The EURODIAB study showed an association with hyperglycaemia and coronary heart disease only in men with type 1 diabetes (210). In the WESDR study, glycaemic control was independently associated with overall CVD mortality but not with myocardial infarction (211), and in the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study cohort, there was no association between hyperglycaemia and coronary heart disease (212). However, at a later stage, an association was found with CVD mortality (215). Finally, data from the DCCT/EDIC demonstrated that a mean of 6.5 years of intensive therapy with a mean HbA_{1c} of ~7% compared with conventional therapy with an HbA_{1c} of ~9% was associated with a 42% risk reduction in all cardiovascular events. Moreover, there was a 57% reduction in the risk of non-fatal MI, stroke or death from CVD after an additional 11 years of observational follow-up in the EDIC study, independent of DN (213). Hyperglycaemia measured as the mean HbA_{1c} was a clear risk factor for CVD events, even after 27 years of observational follow-up, suggesting the importance of early intensive glycaemic management of CVD events (214).

Other risk factors for CVD in type 1 diabetes include hypertension, dyslipidaemia, obesity, insulin resistance, inflammation and smoking, which are also classical risk factors for CVD in type 2 diabetes and the general population. In addition, age and diabetes duration also play a significant role in the development of macrovascular disease. The reduction of the risk of macrovascular disease requires a multifactorial treatment strategy, including smoking avoidance, optimal glycaemic, blood pressure, weight and lipid control, as well as the use of targeted medical agents like RAAS-blockers and statins. (201)

2.3 Physical activity

2.3.1 Definition of physical activity

The most widely used definition of physical activity, written by Caspersen et al. in 1985, is “any bodily movements produced by skeletal muscles that result in energy expenditure” (216). There are various ways to categorise physical activity. One simple approach is to classify physical activity according to the different sections of general daily life: occupational, transportational and leisure-time physical activity (LTPA), i.e. physical activity performed during leisure or discretionary time. LTPA can be further divided into categories such as sports, household tasks etc. Although these terms are often confounded, exercise is a subset of physical activity (216) and refers to any planned, structured and repetitive physical activity with the objective of improving fitness, health or performance (217). Physical activity may improve

physical fitness, which is generally considered the ability to function efficiently and effectively in various activities and emergency situations. It consists of health-related attributes, such as body composition, flexibility, cardiorespiratory and muscular endurance and strength (216). Activities have different components including type, frequency, intensity and duration (218). Physical activity is usually classified as either anaerobic or aerobic, depending on the dominant energy system used for energy supply. Aerobic exercise generally includes low-to-moderate intensity activities that are supported by aerobic fuel metabolism, such as jogging, swimming and cycling. Anaerobic exercise, such as weight lifting or sprinting, includes short, high-intensity activities that do not depend on oxygen as an energy source. However, most activities include a combination of both aerobic and anaerobic energy systems. (217)

2.3.2 Assessment of physical activity

As clear evidence of an association between physical activity and the inverse risk of many chronic diseases is continuously growing, the need and interest in physical activity assessment among different research fields has increased. Contrary to other documentable or measurable risk factors such as hypertension or smoking, physical activity is relatively challenging to measure in comparison. Over 30 different ways of estimating and measuring physical activity are and have been used in research, and no consensus of a gold-standard method exists. Heterogeneous methodology also leads to challenges when studies are compared and their results interpreted.

Daily energy expenditure depends on basal metabolic rate (50–70%), the thermic effect of food (10%) and the most variable component: physical activity. Energy expenditure is generally expressed as kilocalories (kcal) or by using the metabolic equivalent (MET) of the activity, which is defined as multiples of the energy expenditure at rest. The basal rate of oxygen consumption and associated caloric cost is approximately 3.5 ml/kg/min, or about 1 kcal/kg/h, which is assumed to be 1 MET; other activities can be expressed as multiples of the basal 1 MET. (218) However, the MET values also have limitations: age and obesity alter the metabolic rate. For example, children consume more oxygen relative to their body mass at rest, whereas in the elderly, the basal metabolic rate is typically lower (219). The doubly labelled water (deuterium and oxygen-18) technique is considered the most precise method for assessing the energy expenditure of physical activity (220). Its use has increased vastly since 1982, when the first study using this technique in humans was published. The technique is based on the estimation of the rate of carbon dioxide elimination from the body (221). However, the method is very costly and therefore not applicable to large study populations (220).

Table 2: Examples of MET values for different types of activities and recommended intensity level category according to the "Position statement on physical activity and exercise intensity terminology" by K. Norton et al. Table modified from Norton K. et al., *Journal of Science and Medicine in Sport* (2010) (222) and Ainsworth B.E. et al., *Medicine & Science in Sports & Exercise* (2011) (223).

MET level	Example activities	Intensity category
<1.6 METs	Standing in line, watching TV, riding in a car	Sedentary
1.6<3 METs	Household walking, playing darts	Light
3<6 METs	Mopping, vacuuming, low-impact aerobics, brisk walking	Moderate
6<9 METs	High-impact aerobics, basketball, horse racing (galloping)	Vigorous
9<11 METs	Rope jumping (moderate), kickboxing, water polo, soccer (competitive)	High
11<16 METs	Skin diving, vigorous stationary rowing (200W), running 8 mph	
≥16 METs	Running >10 mph, bicycling >20 mph (racing, not drafting)	

Physical activity assessment tools and methods can be classified as subjective or objective. Subjective methods include recall questionnaires and diaries or logs, in which an individual records the activities just after they are performed. There are numerous questionnaires used in research, varying both in question detail and recall time, ranging from hourly to life-time frames. These self-report methods are non-invasive and are mostly used in large epidemiological studies for practical and economic reasons. (218,219) However, these subjective questionnaires have limitations such as human recall and social desirability bias (219). Activity diaries aim to minimise memory errors, but they might increase physical activity above habitual levels during the examination period (220). Therefore, questionnaires with activity recall for longer periods of time might better reflect the habitual activity of an individual (218-220, 224, 225).

Objective methods to measure physical activity are usually based on different devices or applications, including motion sensors such as accelerometers and pedometers, heart rate monitors, direct observation, indirect calorimetry and doubly labelled water (219, 220). Novel objective measurement devices utilise multiple parameters to measure physical activity (225). Objectively measuring physical activity is regarded to be more accurate than subjective methods, but it is also more expensive and therefore not feasible in larger settings. In large study populations, objective methods are mainly used in subsets to validate the

questionnaire used (219). It is noteworthy that all devices and methods share potential biases of increased activity during the measurement period (218-220, 224, 225). Interestingly, modern technology has provided a novel and potentially feasible possibility of objective measurement: e.g. a recent study used smartphones with built-in accelerometry to measure physical activity patterns in different populations worldwide (226).

2.3.3 Physiology

Energy supply is the prerequisite for basic metabolism and physical activity. The body has the ability to convert chemical energy derived from food (carbohydrates, fats and proteins) and sufficient oxygen delivery to adenosine triphosphate (ATP), a molecule that is stored mainly in the muscle cells. During muscle contraction, the required energy is provided by ATP and is broken down by myosin ATPase. The skeletal muscle can increase its energy consumption over 100-fold in response to contraction. This energy is derived mainly from liver glycogen, adipose tissue triacylglycerol and local muscle stores of glycogen and triacylglycerol. There are three main metabolic pathways that provide a continuous supply of ATP for muscle contraction. Interestingly, these systems overlap, and the dominating system depends on the intensity and duration of the activity. The first system is anaerobic and uses ATP and phosphocreatine as energy sources. This is important for the onset of exercise, as it is sufficient enough to support intense contractions just for the first seconds, before other metabolic pathways take over. As exercise continues, anaerobic glycolysis converts muscle glycogen to glucose and then to lactate, providing quick energy but a relatively low ATP yield. Therefore, the muscle glycogen stores are quickly depleted, which limits intense activity duration to approximately 1 minute. The lactate acid leads to a drop in pH levels, resulting in the impairment of muscle contraction and muscle fatigue, felt as “burning”. The third oxygen-dependent pathway, following an increased blood flow and oxygen delivery, utilises glucose, lactate and fatty acid oxidation. This yields ATP for long-term aerobic activities. (227)

The main sources for energy during muscular exercise are fat (triglyceride) and carbohydrate (glycogen and glucose). The contribution of protein as a fuel is relatively small. The proportion of fat vs. glucose utilisation varies greatly due to many factors, e.g. meal timing and consistency before exercise, fitness level, glycogen stores, duration and intensity of exercise. Fat is an important fuel during light to moderate exercise, providing at least half the energy. In prolonged exercise, this proportion can increase up to 80%. Carbohydrate is increasingly vital during high-intensity exercise or bouts. It is also important in energy and exercise metabolism, sparing protein breakdown, functioning as a primer in fat utilisation and as an energy source for the brain. The glucose source for muscles is glycogen from muscle and liver stores and from circulating blood glucose derived mainly from the liver.

During activity, the muscles increase their glucose uptake up to 40-fold, while other tissues decrease their glucose uptake. (227)

The skeletal muscle is insulin sensitive. However, the increased glucose uptake is elevated due to both insulin-dependent and insulin-independent mechanisms (228). Physical activity increases the recruitment of glucose transporters to the surface of muscle cells. This process is stimulated by both insulin and muscle contractions through distinctive signalling pathways, and these stimuli have a cumulative effect (229). The increased glucose uptake should be balanced by hepatic glucose output, increasing gluconeogenesis and glycogen mobilisation to keep the blood glucose at an adequate level. In addition, free fatty acids in the blood and lipolysis are increased.

The main hormonal responses to maintain plasma glucose include increased levels of catecholamines (epinephrine and norepinephrine), glucagon, cortisol, growth hormone and a decrease in insulin release. The responses vary substantially according to the intensity and duration of the exercise. These hormonal responses are discussed in more detail in chapter 2.4.1.

2.3.4 Physical activity: health benefits and the risks of inactivity

It is well documented that physical activity leads to various significant health benefits in both primary and secondary prevention of several chronic diseases and mortality. The first studies are from the 1950s, when Morris et al. showed that the incidence of CAD was higher among sedentary drivers than among more physically active bus conductors (individuals collecting fares and selling tickets) (230). In the following decades, Paffenbarger and colleagues expanded these results (231–233).

In recent years, there has been a growing amount of evidence about the effect of physical activity on the primary and secondary prevention of CVD. The results of a recent meta-analysis and review are in line with the previous evidence. Both LTPA and occupational physical activity reduce the risk of incident CAD and stroke by 10–30% (234). In addition, the benefits of physical activity and fitness also apply to patients with established CVD (7). Regarding all-cause and cardiovascular death, observational data show that physical activity and a high fitness level are associated with a reduced risk of mortality, and there seems to be a dose-response relationship (7). However, it is noteworthy that the findings are mainly limited to Caucasian individuals in Western countries (233).

In general, a higher amount of physical activity is positively associated with the key risk factors for CVD, many of which are mutual to other chronic diseases, as indicated in the pathogenesis of diabetic complications. Physical activity is beneficial for lowering blood pressure and for improvement of the lipid profile (235). Evidence shows that physical activity also improves insulin sensitivity (236), endothelial function (237), glucose homeostasis (238) and autonomic function (239), and it decreases blood coagulation (240) and inflammation (241). Physical activity has

been shown to decrease both body fat and weight and improve fat distribution (235). In addition, other health benefits of physical activity include reduced risk for osteoporosis and cancer, improvement of mental well-being and prevention of cognitive decline (7, 242, 243).

Regarding type 2 diabetes, there is solid evidence about physical activity in both primary and secondary prevention (8–10, 244). Randomised control trials have shown that lifestyle interventions, including physical activity and dietary intervention, provide a substantial benefit leading to disease delay and potential disease prevention (245). Also, exercise interventions have shown benefits regarding glucose control in individuals with T2D (246), and observational studies have shown an association with reduced mortality in these individuals (247). Further, physical activity interventions have helped to reduce cardiovascular risk factors, contribute to weight loss and improve the well-being in individuals with T2D (248, 249).

Despite the well-known and wide-ranging health benefits of physical activity, physical inactivity has become one of the leading public health problems of the 21st century. According to the WHO, more than 80% of the world's adolescent population is insufficiently active, and inactivity is one of the leading risk factors for death worldwide. Physical inactivity caused over 5.3 million deaths, i.e. 9% of premature mortality, worldwide in 2008 (250). In addition, prolonged sedentary time has raised concerns over the years. A recent study by Diaz et al. showed that uninterrupted bouts of sedentary time were associated with all-cause mortality, and prolonged sitting has been shown to be a health risk distinguishable from overall physical activity (251, 252).

Health-related physical fitness involves different components: cardiovascular fitness, musculoskeletal fitness, body composition and metabolism. It refers to a physiologic state, and it can be measured more accurately than physical activity, which is behavioural. Both physical activity and fitness lead to significant health benefits, but evidence suggests that fitness is a more significant predictor of health benefits than physical activity. (253, 254) Interestingly, both also have a genetic component. First, the body's responses to physical activity vary among individuals, and this is at least partly explained by genetics. Fitness is also influenced by genetics (255); for example, VO2max does not increase equally in response to exercise among people (256). Therefore, to reach a certain fitness level, an individual might have to do significantly more activity than another. Second, twin studies have shown that participation in physical activity has a genetic component that is higher for high-intensity activity than for moderate (257–260, 260). Furthermore, as genetics plays a certain role in physical activity and fitness, it also impacts the susceptibility to chronic diseases and disease risk factors, and these genes may overlap. Nevertheless, genetics is not a target for health interventions, and randomised control trials show substantial health benefits of physical activity (7). Furthermore, a study from the

Finnish Twin Cohort showed that LTPA is associated with reduced mortality, even after adjustment for genetic and other familial factors (261).

2.4 Physical activity and type 1 diabetes

2.4.1 Physical activity, hormonal responses and blood glucose in type 1 diabetes

The maintenance of an optimal glucose balance with physical activity requires knowledge of the metabolic and hormonal responses related to type 1 diabetes and the external administration of insulin. On top of this, a lot of information and know-how are required to adjust and optimise insulin doses and food intake with alternating metabolic circumstances related to physical activity. The metabolic and blood glucose responses to exercise vary depending on the duration, intensity, timing and type of the activity (262). Also, other factors contribute to the highly variable glucose responses. For example, meal consistency and timing, the site of insulin injections, the peak action of insulin, the starting glucose levels, prior exercise or hypoglycaemia, stress level, environmental factors such as temperature and altitude, individual fitness and menstrual cycle may all alter the glucose response to activity (262).

In non-diabetic subjects, insulin secretion is reduced during prolonged aerobic activity, while the glucagon secretion increases. This induces glycogenolysis and gluconeogenesis in the liver, which facilitates glucose supply for the working muscles. As the duration of physical activity is prolonged, the muscle glycogen stores are depleted. Consequently, the glucose output from the liver and an activated lipolysis also augmented by other counterregulatory hormones (mainly catecholamines, cortisol and growth hormones (GH)) become more important as energy sources. (263) In individuals with type 1 diabetes, the physiological reduction in insulin secretion is absent, and the amount of circulating insulin depends on the dose and timing of the exogenous insulin administration (264). In addition, physical activity increases blood flow in the subcutaneous tissue, thereby also increasing the absorption of insulin. Typically, without modulation of insulin dosing, prolonged activity leads to a progressive decline in blood glucose. This relative excess of insulin also affects the utilised fuel sources. Lipolysis may decline, and the muscles will therefore be more dependent on glucose. In addition, high relative insulin levels lead to decreased glucose disposal from the liver, increased muscle glucose uptake and decreased glycogen utilisation. (265)

On the contrary, if exercise is initiated during insulin deprivation and hyperglycaemia, there is a substantial risk that exercise will further elevate the glucose levels due to inappropriate counterregulatory responses and fuel metabolism, and this may even lead to severe ketosis (264). In addition, aerobic exercise during

hyperglycaemia increases the rate of carbohydrate oxidation over lipid oxidation compared to exercise during physiological conditions with normal glycaemia. On the other hand, during near physiological insulin concentrations and euglycaemic conditions, the fuel metabolism is similar between individuals with type 1 diabetes and healthy subjects. (266)

Higher-intensity activities increase the proportion of glucose compared to lipids as a fuel source (267). Therefore, especially prolonged and intensive exercise increases the carbohydrate demand, which must be considered by individuals with type 1 diabetes before and during exercise. Generally, guidelines recommend insulin dosage reduction before, during and after exercise, and/or extra carbohydrate consumption due to increased insulin sensitivity and carbohydrate demand (268). On the other hand, in short anaerobic activities, the catecholamines increase blood glucose levels, which is accompanied by a quick increase in blood insulin levels in healthy individuals (269). Also, in shorter, partly anaerobic high-intensity interval sessions, there is less reduction in insulin levels than in prolonged aerobic activities. After a high-intensity interval session, insulin levels increase in order to counteract changes in metabolites and the increased counterregulatory hormone responses to prevent hyperglycaemia. Therefore, an individual with type 1 diabetes might need an extra bolus of insulin to prevent hyperglycaemia related to intense activities. (270) However, it is important to exercise with caution in order to avoid post-exercise hypoglycaemia due to increased insulin sensitivity and replenishment of glycogen stores (267).

The main adverse event related to physical activity in type 1 diabetes is hypoglycaemia. In fact, the fear of severe hypoglycaemia is the most significant barrier to exercising for many individuals with type 1 diabetes (271). First, the increase in post-exercise insulin sensitivity is an important reason that hypoglycaemia lasts up to 24–48 h following the exercise (263, 265). The risk for hypoglycaemia after physical activity is higher for at least 24 hours (263). In addition, studies have shown that the normal counterregulatory hormone response may be deficient in individuals with type 1 diabetes. Previous hypoglycaemic events impair this response even further, resulting in a greater likelihood of further hypoglycaemic events. Interestingly, even in healthy individuals, hypoglycaemia diminishes counterregulatory responses to exercise. (265) In type 1 diabetes, this antecedent hypoglycaemia-induced autonomic failure is a significant mechanism resulting in inadequate endogenous glucose production and hypoglycaemia (272). In addition, autonomic neuropathy causes hypoglycaemia unawareness, and it further attenuates the sympathetic response to hypoglycaemia after previous hypoglycaemia, leading to a potential vicious cycle of hypoglycaemic events (273). Moreover, it has been shown that physical activity itself may decrease the counterregulatory response to hypoglycaemia (274).

Physical activity improves glycaemic control (measured by HbA_{1c}) in type 2 diabetes, and this has been shown in a variety of different forms of exercise, such

as aerobic and resistance training (275). However, in type 1 diabetes, the data are inconsistent, and there is no clear established benefit. A meta-analysis by Kennedy et al. showed no clear benefit of exercise on glycaemia in individuals with type 1 diabetes. It is however of note, that the data demonstrated that physical activity can be undertaken safely without significant hypoglycaemia. (276) This is important since physical activity provides significant benefits in type 1 diabetes, in which hypoglycaemia is the main barrier to exercising (271). In adolescents, however, it seems that physical activity is associated with better glycaemic control. A recent meta-analysis of 10 trials in individuals below 18 years of age showed an overall significant benefit of physical activity on glycaemic control, measured as HbA_{1c} (277).

2.4.2 Physical activity and type 1 diabetes: health benefits and impacts on complications

The consensus is that physical activity is beneficial for an individual with type 1 diabetes and is therefore recommended by the guidelines. However, research data on the matter are surprisingly limited, especially regarding diabetic complications and mortality, not to mention the lack of exercise intervention studies. Most of the guidelines are based on knowledge obtained from type 2 diabetes and the general population. There is evidence that complication-free and young individuals with type 1 diabetes are as physically active as their peers (278). As we know, most youth do not meet the general recommendations for activity, so these levels are not optimal. Adults with type 1 diabetes are less active and have lower fitness levels than their non-diabetic peers (271, 278, 279).

Data on the impact of physical activity on diabetic complications and mortality in type 1 diabetes are scarce. However, studies implicate that physical activity benefits many risk factors associated with the development of diabetic complications and mortality in type 1 diabetes. Several studies have shown the benefit of physical activity on the lipid profile by increasing the amount of high-density lipoprotein cholesterol and decreasing the low-density lipoprotein cholesterol and triacylglycerol levels (280–282). Also, physical activity decreased the level of apolipoprotein B (proatherogenic), whereas a polipoprotein A-1 (which protects against atherosclerosis) was increased (283, 284). Endothelial dysfunction is associated with cardiovascular events and is more common in individuals with type 1 diabetes. Studies have shown that physical activity improves endothelial function in these individuals (285–287). Regarding hypertension, there are only a few studies assessing the association of physical activity with blood pressure in individuals with type 1 diabetes, and most of these studies have not shown a clear benefit; however, the number of participants in these studies has been relatively small (282, 287, 288). In contrast, a study by Lehmann et al. showed a statistically

significant reduction in both systolic (SBP) and diastolic blood pressure (DBP) after a three-month exercise intervention (289).

Several studies have shown that physical activity improves fitness in individuals with type 1 diabetes (287, 290, 291). Also, physical activity improves well-being: self-reported physical activity was associated with a better quality of life in individuals with type 1 diabetes (292).

Traditionally, type 1 diabetes has been considered a disease of the lean and is characterised by an absolute insulin deficiency. However, the prevalence of obesity has increased in type 1 diabetes, and insulin resistance is independently associated with an increased risk of micro- and macrovascular complications (293–295). In addition, insulin resistance may be accompanied by the clustering of other CVD and metabolic risk factors, such as elevated blood pressure, worse lipid profile, inflammation and visceral fat (296, 297). Several studies have shown that physical activity improves insulin sensitivity (281, 287, 291). Moreover, Ramalho et al. showed that both aerobic and resistance training improves insulin sensitivity (298).

Due to the beneficial effects of several risk factors, it can be assumed that physical activity might reduce the risk of diabetic micro- and macrovascular complications. Previous cross-sectional studies have shown that more physically active individuals with type 1 diabetes have fewer signs of diabetic nephropathy, neuropathy and retinopathy than less active individuals (299, 300). In addition, we previously showed that individuals with type 1 diabetes and microalbuminuria reported undertaking physical activity of lower intensity compared with individuals without albuminuria (300). The study design was cross-sectional, and the result could be due to the fact that complication-free individuals are more likely to engage in physical activity, but on the other hand, people with microalbuminuria usually have no symptoms and may not be aware of it.

Prospective data regarding physical activity and its association with diabetic complications and mortality are very limited in type 1 diabetes. To our knowledge, no studies have assessed the relationship between physical activity and the development and progression of renal disease in type 1 diabetes using a prospective study design. So far, the strongest evidence regarding physical activity and macrovascular disease and mortality derives from the Pittsburgh IDDM Morbidity and Mortality study. Men who participated in team sports during high school were less likely to die prematurely or to have macrovascular disease after having type 1 diabetes for 25 years (301). Also, retrospective information on physical activity at 14–17 years of age was associated with less neuropathy and nephropathy. However, this was found in men only, and physical activity during adolescence was not associated with severe retinopathy or blindness (299). Furthermore, these individuals were followed during adulthood, and overall physical activity was assessed by a standardised questionnaire. The level of physical activity was inversely associated with mortality, even after controlling for a number of potential confounders (age, BMI, insulin doses,

diabetic complications, smoking, current alcohol consumption). Again, however, this association was statistically significant only in men. (278) In addition to the Pittsburgh study, the EURODIAB study reported a borderline inverse association between physical activity and all-cause mortality when both sexes were combined (302). Regarding CVD, a borderline inverse association was found only in women. Moreover, the landmark Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that participation in high school team sports was associated with lower incidence of diabetic retinopathy, but only in a subgroup of women diagnosed with diabetes under the age of 14 (303).

2.4.3 Recommendations of physical activity in type 1 diabetes

The recommendations for physical activity and diabetes have recently been updated in the ADA position statement for diabetes and physical activity/exercise (268). The weekly minimum amount of physical activity recommended for most adults with diabetes includes 150 minutes of physical activity with an intensity level of moderate-to-vigorous. This amount should be divided into at least three different days per week, and there should be no more than two consecutive days without physical activity. More than 75 minutes of interval training or vigorous activity per week might be sufficient for individuals that are more physically fit. In addition, 2–3 resistance training sessions should be performed every week in non-consecutive days, and for older adults, 2–3 flexibility and balance training sessions are also recommended. Additionally, an individual with diabetes should reduce daily sedentary time. Long-term sitting should be interrupted every 30 minutes with bouts of light activity. This does not replace the other recommended amount of weekly physical activity. (268) The recommendations for physical activity for type 1 diabetes are very similar in the Finnish Current Care guidelines (5).

As discussed earlier, the glucose responses to exercise are affected by many factors, and therefore, specific and uniform recommendations for exercise-related glycaemic control is not possible. Frequent blood glucose measurements, however, are necessary for adjusting the optimal carbohydrate amount and insulin dosing. Exercise is not recommended during hyperglycaemia or elevated blood ketones. Individualised patient education is crucial for optimising insulin dosages that depend on blood glucose levels and the type of physical activity engaged. Specific medical clearance before engaging in light to moderate physical activity is usually not needed for an asymptomatic patient beyond normal guideline-conducted diabetes care (268). However, if an individual with diabetes has complications or is increasing the intensity level of physical activity, examination and approval by a physician is recommended, and in some cases, an exercise stress test is required before engaging in physical activity (5, 268).

3 AIMS OF THE STUDY

The data regarding physical activity and diabetic complications and mortality in type 1 diabetes are limited. The main objective of this study, therefore, was to investigate the role of LTPA in the development of diabetic complications and mortality in individuals with type 1 diabetes in a prospective study design.

Specifically, the aims of the present study were:

I To examine the relationship between physical activity and the development and progression of diabetic nephropathy in individuals with type 1 diabetes.

II To assess whether physical activity is associated with the risk of incident or recurrent CVD events in individuals with type 1 diabetes.

III To evaluate the association between physical activity and all-cause and CVD mortality in individuals with type 1 diabetes with and without CKD.

IV To investigate the association between physical activity and the development of severe diabetic retinopathy in individuals with type 1 diabetes.

4 SUBJECTS AND STUDY DESIGN

4.1 The FinnDiane Study

The individuals included in these studies are participants of the ongoing nationwide and multi-centre Finnish Diabetic Nephropathy (FinnDiane) Study that was launched in 1997. The FinnDiane Study aims to identify clinical, biochemical, genetic and environmental risk factors for diabetic complications, with an emphasis on diabetic nephropathy. So far, over 5000 adult individuals with type 1 diabetes – that is, more than 10% of the total number of individuals with type 1 diabetes in Finland – have been recruited and thoroughly characterised. The distribution of the FinnDiane patients is shown in Figure 2. The study centres include all 5 university hospitals, all 16 central hospitals, 26 regional hospitals and 30 primary healthcare units (Appendix).

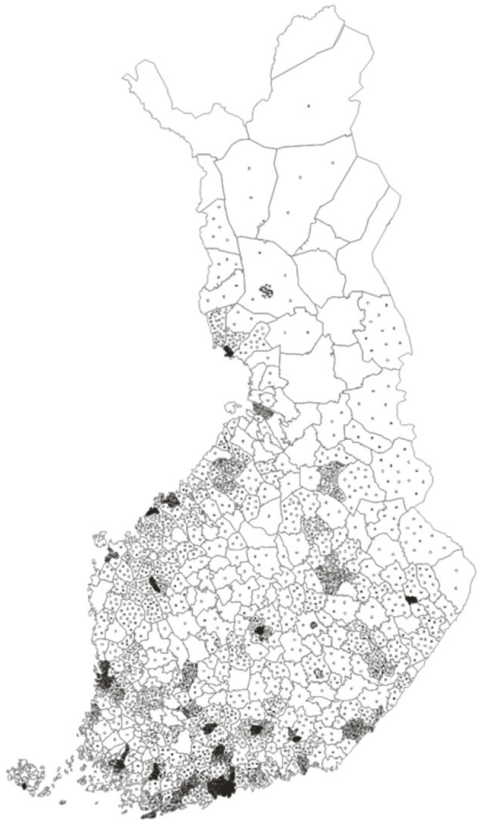


Figure 2. Geographical distribution of the FinnDiane patients. Each dot indicates one FinnDiane patient. The distribution is similar to the distribution of the general Finnish population.

The LTPA questionnaire was introduced at the beginning of the year 2000, and in this thesis, all participants with available data on baseline LTPA were included. Until 2011, a total of 62% of the participants in the FinnDiane Study had answered the questionnaire. The clinical characteristics of the questionnaire respondents were comparable to those that did not participate. None of the participants had comprehensive data available for all the different LTPA components, but we aimed to use the maximum amount of available data. Therefore, the total number of patients differed somewhat in the analyses of total amount, intensity, frequency and duration of LTPA. Follow-up data have been collected since 2004. The study design of this thesis was prospective and observational. The inclusion criteria for the participants in each study is shown in Figure 3.

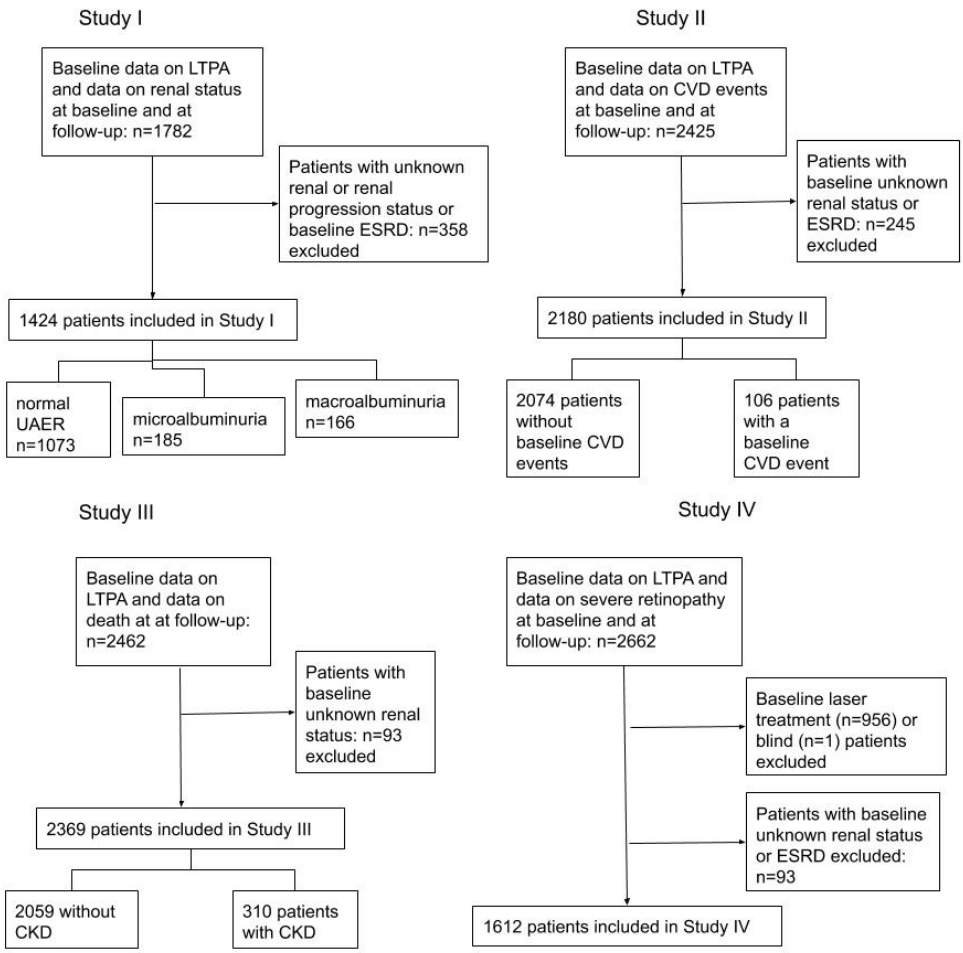


Figure 3. Flow chart of patient selections: Studies I–IV.
ESRD: End-stage renal disease, CVD: Cardiovascular disease, UAER: Urinary albumin excretion rate, LTPA: Leisure-time physical activity, CKD: Chronic kidney disease.

4.2 Study population: clinical characteristics (Studies I–IV)

The inclusion criteria for the study population in each study is shown in the Methods section, 4.1 Figure 3. The baseline clinical characteristics of the study population in each study are shown in Table 3.

Table 3. Clinical characteristics of the study population (Studies I–IV)

	Study I	Study II	Study III	Study IV
N	1424	2074	2639	1612
Male (%)	48.5	47.2	48.5	44.7
Duration of diabetes (years)	20.4 ± 12.3	21.7 ± 12.4	23.3 ± 12.8	18.9 ± 11.7
Age (years)	37.0 ± 12.4	38.8 ± 12.4	40.1 ±12.6	37.0 ± 11.9
BMI (kg/m^2)	25.0 ± 3.4	25.2 ± 3.6	25.2 ± 3.6	25.1 ± 3.6
SBP (mmHg)	132 ± 16	133 ± 17	135 ± 19	131 ± 16
HbA_{1c}	8.3 ± 1.4% (67 ± 15 mmol/mol)	8.3 ± 1.4% (67 ± 15 mmol/mol)	8.3 ± 1.4% (67 ± 15 mmol/mol)	8.2 ± 1.4% (66 ± 15) mmol/mol
Current or previous smokers (%)	42.6	44.4	44.8	41.5
Normal UAER (%)	75.4	74.9	68.9	87
Sedentary (%)	29.1	33.2	31.6	29.9

4.3 Ethical aspects

The study protocol was approved by the ethics committees of all participating centres as well as the Ethics Committee of the Helsinki and Uusimaa Health Region. Written informed consent was obtained from each patient prior to participation, and the study was performed in accordance with the Declaration of Helsinki.

5 METHODS

5.1 The FinnDiane Study protocol

During a regular visit to the attending physician, data on thorough medical history, medication, cardiovascular status and diabetic complications were recorded by a standardised questionnaire and completed and verified through the medical files. Information on the history of smoking and current alcohol consumption was assessed by standardised questionnaires. The regular visits also included urine collections, blood samples for laboratory measurements and various assays, measurements of anthropometric parameters and blood pressure (121).

5.1.1 Definition of type 1 diabetes

Type 1 diabetes was defined as the onset of diabetes before the age of 35 (Study I) or 40 years (Studies II–IV) and with insulin treatment initiated within 1 year of diagnosis.

5.1.2 Definition of diabetic nephropathy and assessment of renal function

Renal status was categorised based on local and central measurements UAER in either a timed overnight or 24-h urine collection in at least two out of three consecutive measurements. Renal status was categorised as follows: normal UAER <20 μg/min or <30 mg/24 h; microalbuminuria ≥20 and <200 μg/min or ≥30 and <300 mg/24 h (n=295); or macroalbuminuria ≥200 μg/min or ≥300 mg/24 h. Participants requiring dialysis or having received a kidney transplant were classified as having end-stage renal disease (ESRD). Diabetic nephropathy was defined as macroalbuminuria or ESRD. Individuals with unknown renal status were excluded. We assessed the progression of diabetic nephropathy during follow-up in Study I, and renal progression was defined as any shift to a higher albuminuria class or the development of ESRD. Renal function (estimated glomerular filtration rate [eGFR]) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) (66). In Study III, individuals with eGFR ≤60 mL/min/1.73 m2 or ESRD were classified as having CKD. However, individuals with a kidney transplant and eGFR >60 mL/min/1.73 m2 (n=53) were not defined as having CKD. In addition, patients with CKD were classified as having CKD without ESRD, undergoing dialysis or having a kidney transplant (Study III).

5.1.3 Definition of CVD

In study I, CVD was defined as a clinically verified myocardial infarction, coronary revascularisation procedure, stroke, ischaemic limb amputation or a peripheral artery revascularisation procedure. In Studies II–IV, a major CVD event was defined as a clinically verified myocardial infarction (ICD-8/9 410–412, ICD-10 I21–I23), coronary procedure (bypass grafting surgery or angioplasty based on the Nordic Classifications of Surgical Procedures) or either an ischaemic or haemorrhagic stroke (ICD-8/9 430–434, ICD-10 I60–I64). Follow-up events of CVD were identified from the Care Register for Health Care (HILMO) and the Finnish Causes of Death Registry and were verified with medical records. The date of the first CVD event was defined as the date of hospital admission due to CVD.

5.1.4 Definition of severe diabetic retinopathy

Severe diabetic retinopathy is defined as the initiation of laser treatment due to severe non-proliferative retinopathy, proliferative retinopathy or diabetic maculopathy, as identified by the Care Register for Health Care (HILMO).

5.1.5 Anthropometric measurements

Anthropometric data were collected by a trained nurse. Body weight was registered to the closest 0.1 kg, and height was registered to the closest 1 cm. The calculation of weight-to-hip ratio (WHR) included dividing the waist circumference (measured midway between the lowest ribs and the iliac crest) by the hip circumference (measured at the major trochanters of the femurs). Body mass index (BMI) was calculated as body weight/height^2 (kg/m^2). Blood pressure was measured twice after a 10-minute rest with a 2-minute interval either by a manual sphygmomanometer or an automated blood pressure measurement device. The mean values of SBP and DBP were calculated and used in the analyses.

5.1.6 Definition of smoking

Data on smoking status were collected by a self-administered questionnaire. Participants were categorised either as current smokers, previous smokers or never smokers. History of smoking was defined as smoking one or more cigarettes per day for at least 3 months and ceasing prior to data collection. Current smoking was defined as smoking at least one cigarette a day during data collection.

5.1.7 Laboratory measurements and assays

Blood samples were drawn to analyse the lipid profile, HbA_{1c} and creatinine. Serum lipids and lipoproteins were measured at the research laboratory of Professor Marja-Riitta Taskinen, Department of Medicine, Division of Cardiology, Helsinki University Hospital, Helsinki, Finland. The Friedewald formula was used to calculate LDL cholesterol (304). The measurement of serum creatinine was performed by a kinetic Jaffé reaction until January 2002 and afterwards by a photometric, enzymatic method (Hitachi 917 or Modular analyser, Boehringer Mannheim/ Roche Diagnostics, Basel, Switzerland). HbA_{1c} was measured locally at each study centre by standardised assays with a normal range of 20–42 mmol/mol (4.0–6.0%). Urinary albumin concentration was measured with RIA until 1 November, 2002, and thereafter by immunoturbidimetry.

5.2 Assessment of physical activity

LTPA was assessed by a baseline self-report questionnaire, introduced at the beginning of 2000, and in this study, we included all individuals with available data on LTPA. The "KIHD 12-month Leisure-Time Physical Activity Questionnaire" is a Finnish conversion of the Minnesota Leisure-Time Activity Questionnaire that was validated against double-labelled water (305, 306). The KIHD questionnaire has been validated in 1163 Finnish men from the general population, with maximum oxygen uptake as the standard method validation (307).

The questionnaire is a detailed quantitative questionnaire assessing duration, frequency and mean intensity of the most common lifestyle and structured LTPA in Finland, as recalled over the previous 12 months. The first section of the questionnaire contains questions of general type, frequency, duration and intensity of LTPA. Information about general activity duration, frequency and intensity was used from the first section of the questionnaire. Additionally, a second section asks specific details on frequency (times per month), duration per session and intensity for 21 types of predefined activities retrospectively from the past 12 months. Based on the intensity level (0–3), each of the 21 activities is assigned a specific metabolic equivalent (MET) value. In addition, the questionnaire contains a third section that assesses physical activity at work. However, the amount of data in this section was limited and was therefore not used in the analyses in this thesis.

The studies considering this questionnaire imply that it is representative and shows relatively small intra-person variability, and the 12-month LTPA correlated with the VO_{2max} (308). The classification of the sedentary level of LTPA (<10 METh/ week) was defined as an LTPA lower than the minimum given by general physical activity guidelines (309). For the moderately active and active threshold levels,

we used an arbitrary cut-off, which we have used in our previous publications (moderately active 10–40 METh/week and active >40 METh/ week) (300, 310). We also analysed other LTPA-related exposures: (1) exercise intensity (*low:* no self-reported subjective shortness of breath and no sweating, *moderate:* a moderate degree of self-reported subjective shortness of breath and sweating, and *high:* a high degree of subjective shortness of breath and sweating); (2) single session duration (*low:* ≤30, *moderate:* 31–60 and *high:* >60 min/session); and (3) exercise frequency (*low:* <1, *moderate:* 1–2, and *high:* >2 sessions/week). The classification of LTPA and its components are shown in Table 4.

Table 4. Classification of LTPA and its components

	LTPA	Intensity	Frequency	Duration
Low	<10 METh/week	no self-reported subjective shortness of breath and no sweating	<1 session/week	≤30 min/session
Moderate	10–40 METh/week	a moderate degree of self-reported subjective shortness of breath and sweating	1–2 sessions/week	31–60 min/session
High	>40 METh/week	a high degree of subjective shortness of breath and sweating	>2 sessions/week	>60 min/session

5.3 Statistical analyses

The statistical analyses were performed with SPSS Statistics versions 21 and 22.2 (IBM, Armonk, NY, USA) and SAS version 9.3 (SAS Institute, Cary, NC), and the figures were drawn using GraphPad Prism version 5 (GraphPad, La Jolla, CA, USA). Data are presented as means ± SD if normally distributed and otherwise as median and interquartile range (IQR). Categorical variables are given as percentages. ANOVA was used for normally distributed variables, and the Kruskal–Wallis test was used for non-normally distributed variables. For categorical variables, the $\chi 2$ test was used. Trend test p-values were used when appropriate. The cumulative incidence or progression rates were estimated using the Kaplan–Meier method, and the log rank test was used to test between-group differences. Follow-up started from the baseline visit (2000–2013). The person-years at risk were calculated until the progression of renal status, death or the end of 2012 (Study I); the first CVD event, death or the end of 2013 (Study II); death or the end of 2014 (Study III); the first severe diabetic retinopathy event, death or the end of 2015 (Study IV). The association between different LTPA components and the different end points (renal progression, incident CVD event, death or first severe diabetic retinopathy

event) were analysed using univariable and multivariable Cox proportional hazards regression models. The highest category for each LTPA component was used as a reference group in all multivariable analyses, and the results are presented as HRs with 95% CIs. A p-value of <0.05 was considered statistically significant.

6 RESULTS

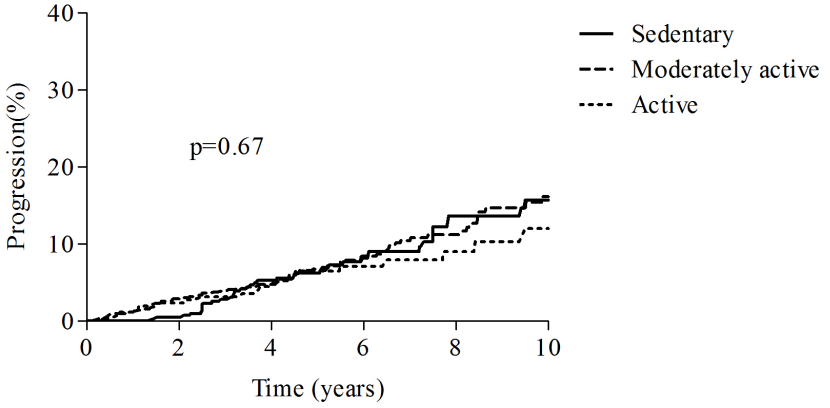
6.1 Study I: The association of LTPA with the development and progression of diabetic nephropathy in type 1 diabetes

In this study, we assessed how LTPA is associated with the development and progression of diabetic nephropathy in individuals with type 1 diabetes. At baseline, physically inactive participants had more often a history of smoking, and they consumed more alcohol and were on antihypertensive drugs more frequently. In addition, individuals with a lower level of LTPA had more abdominal obesity, worse glycaemic control and a higher triacylglycerol level. LTPA was not associated with gender, age or duration of diabetes.

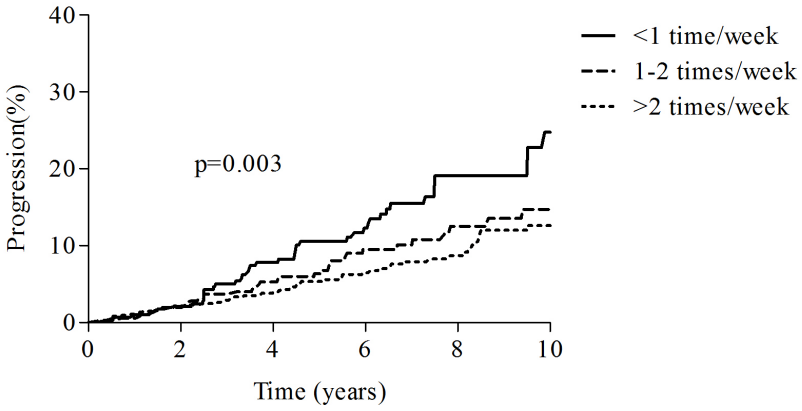
During a follow-up time of 6.4 ± 3.1 years, 149 individuals progressed in their renal status. A total of 72 normoalbuminuric individuals developed microalbuminuria, 35 microalbuminuric individuals developed macroalbuminuria, and 42 macroalbuminuric individuals developed ESRD. At baseline, the subjects that progressed in their renal status were heavier and had higher blood pressure, worse glycaemic control and lipid profile. In addition, progressors were primarily male, previous or current smokers, and they consumed more alcohol.

Figure 4 presents the Kaplan–Meier curves showing the relationship between LTPA and its components with renal progression. The intensity and frequency of LTPA were associated with the progression of renal status: in patients with a low intensity of LTPA, the 10-year cumulative progression rate was 24.0% (95% CI 18.8, 28.8); in those with a moderate intensity of LTPA, it was 13.5% (95% CI 10.3,16.6); and in those with a high intensity of LTPA 13.1% (95% CI 10.3, 16.6) (p=0.01). Also, participants with an LTPA frequency of <1, 1–2 or >2 times per week had a 10-year cumulative risk of progression of renal status of 24.7% (95% CI 18.3, 30.7), 14.7% (95% CI 10.2, 19.0) or 12.6% (95% CI 9.4, 15.7), respectively (p=0.003). The total amount of LTPA and single-session duration, however, were not associated with renal progression.

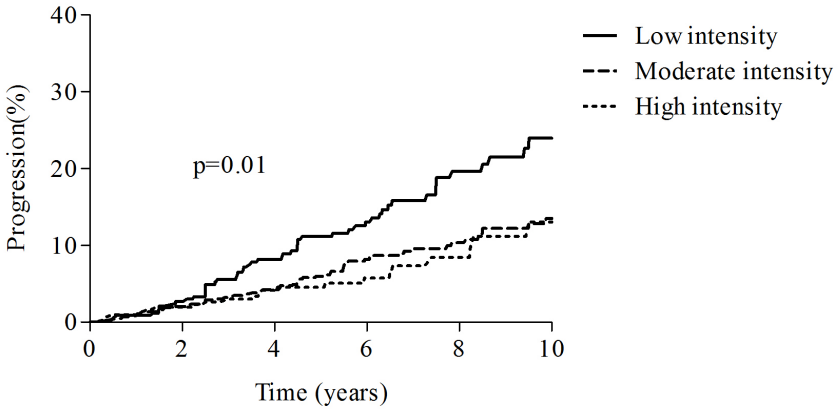
a) LTPA



b) Frequency



c) Intensity



d) Duration

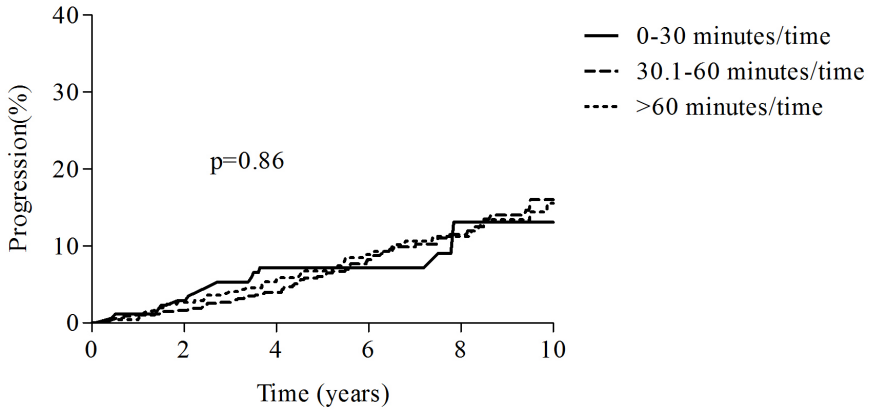


Figure 4. Kaplan–Meier curves for total LTPA and components of LTPA regarding progression of renal status, defined as any shift to a higher albuminuria class or to ESRD.

Adapted by permission from Springer Nature, Diabetologia 2015;58(5):929–936. Copyright © 2015, Springer Nature

Table 4 presents the multivariable analyses of the association of low vs. moderate and high LTPA intensity with progression of renal status. Model 1 is a univariable model including the intensity of LTPA. In model 2, we added static confounders, such as duration of diabetes, sex and current smoking. At this stage, the intensity of LTPA was still associated with renal progression. In contrast, in models 3–6, the confounding factors (HbA_{1c}, BP, triacylglycerol and BMI) that could potentially be influenced by physical activity incrementally decreased this association to a non-significant level. The association with LTPA frequency and renal progression decreased to a non-significant level after adjusting for either static or dynamic confounders.

Table 4. Cox regression models showing hazard ratios for low vs. moderate and high intensity LTPA for progression of renal status

	Progression to micro- or macroalbuminuria	Progression to microalbuminuria	Progression to ESRD
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Model 1	n=1230 1.79 (1.19, 2.69)	n=1049 1.54 (0.92, 2.59)	n=160 1.17 (0.63, 2.17)
Model 2	n=1211 1.58 (1.04, 2.41)	n=1035 1.41 (0.83, 2.41)	n=159 1.20 (0.64, 2.27)
Model 3	n=1198 1.53 (1.00, 2.32)	n=1024 1.31 (0.77, 2.23)	n=154 1.23 (0.62, 2.46)
Model 4	n=1190 1.46 (0.96, 2.24)	n=1016 1.26 (0.73, 2.17)	n=153 1.28 (0.64, 2.56)
Model 5	n=1160 1.43 (0.93, 2.22)	n=989 1.31 (0.76, 2.27)	n=147 1.36 (0.67, 2.79)
Model 6	n=1156 1.48 (0.96, 2.29)	n=986 1.22 (0.70, 2.14)	n=147 1.40 (0.67, 2.94)

Model 1: low-intensity LTPA
Model 2: Model 1 + sex, duration of diabetes and current smoking
Model 3: Model 2 + HbA_{1c}
Model 4: Model 2 + mean arterial pressure
Model 5: Model 2 + triacylglycerol
Model 6: Model 2 + BMI

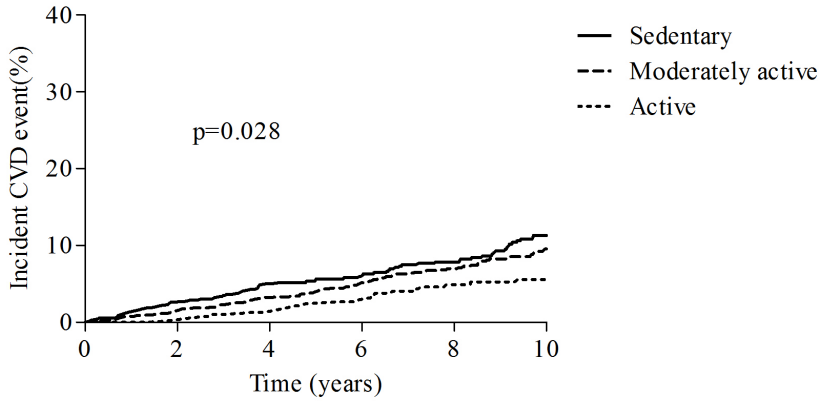
LTPA: leisure-time physical activity.

To assess the possible bias regarding baseline renal disease and physical inactivity, we separately analysed the association of LTPA and its components with the development of *de novo* microalbuminuria (shift from normo- to microalbuminuria). In the Kaplan–Meier models, only LTPA intensity was associated with the development of microalbuminuria: the cumulative progression rate in an 8-year follow-up was 15.4% (95% CI 9.4, 21.0), 6.9% (95% CI 4.5, 9.3) and 5.9% (95% CI 2.1, 9.6) in the low-, moderate- and high-intensity groups, respectively (p=0.047, log rank test). Regarding intensity in this sub-analysis, the finding in the multivariable analyses showed comparable HRs but non-significant p-values after correction for both static and dynamic confounders. For total LTPA, the development of microalbuminuria was significantly higher in sedentary individuals compared with those with moderately active or active levels of LTPA, at 1.90 (95% CI 1.04, 3.49) in the final Cox regression model. LTPA frequency or duration showed no association with the development of microalbuminuria in either the univariable or multivariable models.

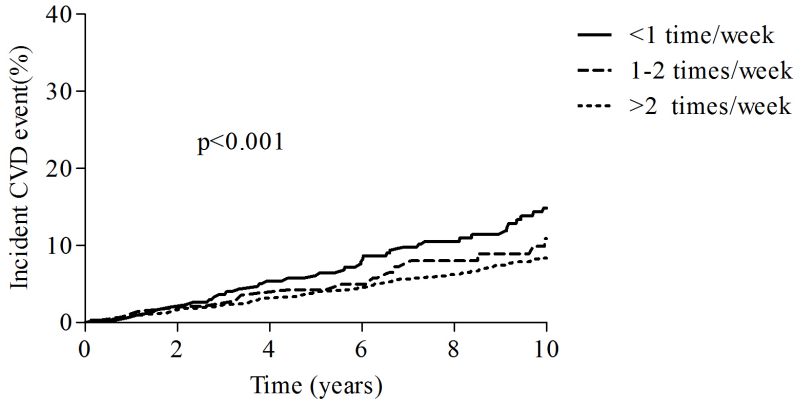
6.2 Study II: The association of LTPA with the development of incident and recurrent CVD events in type 1 diabetes

We made separate analyses for the association of LTPA and its components with the development of an incident and a recurrent CVD event. Of the 2074 individuals without a previous CVD event, 206 (9.9%) had an incident CVD event during a mean follow-up of 10.3±3.4 years. The Kaplan–Meier curves of the association of LTPA and its components with incident CVD are shown in Figure 5. The various components were associated with the development of a first major CVD event: the 10-year cumulative incidence of CVD increased with decreasing amounts of physical activity irrespective of which component was studied. Total LTPA (p=0.028), exercise intensity (p<0.001), exercise duration (p=0.014) and exercise frequency (p<0.001) were all associated with incident CVD.

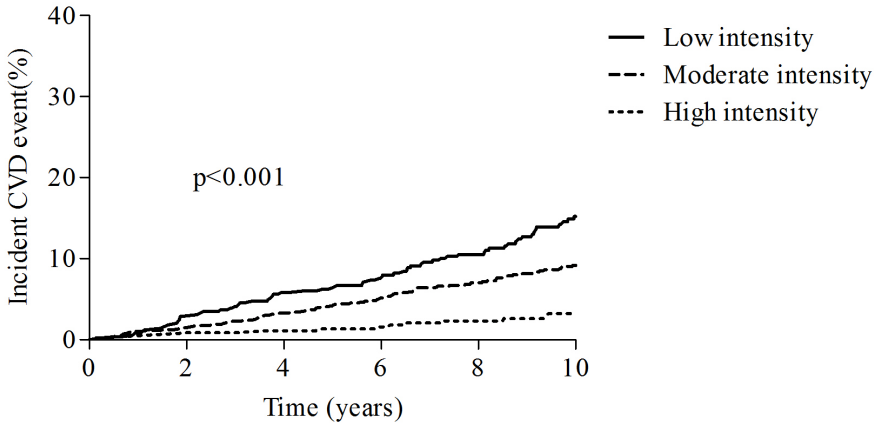
a) LTPA



b) Frequency



c) Intensity



d) Duration

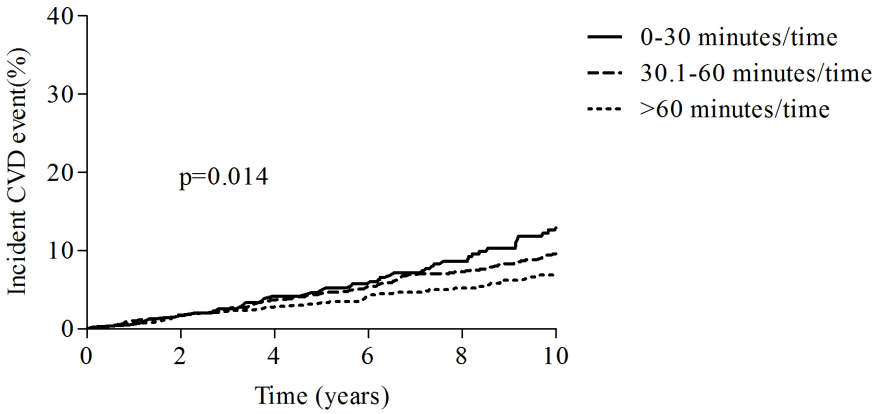


Figure 5. Kaplan–Meier survival analysis for an incident CVD event stratified by total LTPA and components of LTPA. LTPA: Leisure-time physical activity.

Next, we performed the Cox regression analyses. The first model included only the physical activity components. In model 2, we added the static confounders (duration of diabetes, age at onset of diabetes, diabetic nephropathy and gender) to the multivariable analysis. The total amount of LTPA, intensity and frequency were still associated with incident CVD. However, the association with duration decreased to a non-significant level. The final model 7 included all the static and dynamic confounders (triacylglycerol, BMI, SBP, HbA_{1c} and smoking). Only the association with frequency remained significant in the final model. The effect of LTPA intensity was slightly reduced to become non-significant in model 6, while the total amount of LTPA and duration were non-significant in the models including dynamic confounders. The Cox regression models are shown in Table 5.

Table 5. Cox regression models showing hazard ratios for low and moderate vs. high LTPA, intensity, frequency and duration for an incident CVD event

Model 1:	n=2074, 206 events	n=2030, 199 events	n=2055, 206 events	n=2074, 206 events
High	1.00	1.00	1.00	1.00
Moderate	1.41 (0.92, 2.14)	2.58 (1.55, 4.31)	1.17 (0.69, 2.00)	1.27 (0.91, 1.78)
Low	1.77 (1.15, 2.72)	4.51 (2.67, 7.63)	1.91 (1.37, 2.64)	1.76 (1.20, 2.60)
Model 2:	n=2074, 206 events	n=2030, 199 events	n=2055, 206 events	n=2074, 206 events
High	1.00	1.00	1.00	1.00
Moderate	1.51 (0.99, 2.30)	1.49 (0.88, 2.53)	1.56 (0.91, 2.66)	1.17 (0.84, 1.64)
Low	1.59 (1.03, 2.44)	1.91 (1.11, 3.28)	1.94 (1.39, 2.71)	1.37 (0.93, 2.02)
Model 3:	n=2071, 206 events	n=2027, 199 events	n=2052, 206 events	n=2071, 206 events
High	1.00	1.00	1.00	1.00
Moderate	1.50 (0.98, 2.28)	1.45 (0.86, 2.45)	1.52 (0.89, 2.61)	1.16 (0.83, 1.63)
Low	1.51 (0.98, 2.33)	1.77 (1.03, 3.07)	1.84 (1.31, 2.59)	1.29 (0.87, 2.91)
Model 4:	n=2062, 206 events	n=2018, 199 events	n=2043, 206 events	n=2062, 206 events
High	1.00	1.00	1.00	1.00
Moderate	1.50 (0.98, 2.28)	1.46 (0.86, 2.47)	1.52 (0.89, 2.61)	1.16 (0.83, 1.63)
Low	1.51 (0.98, 2.33)	1.81 (1.04, 3.12)	1.85 (1.32, 2.60)	1.30 (0.87, 1.92)
Model 5:	n=2052, 206 events	n=2008, 199 events	n=2033, 206 events	n=2052, 206 events
High	1.00	1.00	1.00	1.00
Moderate	1.50 (0.98, 2.28)	1.47 (0.87, 2.50)	1.48 (0.87, 2.54)	1.21 (0.86, 1.69)
Low	1.49 (0.97, 2.31)	1.83 (1.06, 3.17)	1.78 (1.27, 2.51)	1.30 (0.87, 1.92)
Model 6:	n=2014, 205 events	n=1971, 198 events	n=1995, 205 events	n=2014, 205 events
High	1.00	1.00	1.00	1.00
Moderate	1.42 (0.93, 2.17)	1.41 (0.83, 2.40)	1.41 (0.82, 2.42)	1.20 (0.86, 1.69)
Low	1.38 (0.89, 2.13)	1.68 (0.97, 2.92)	1.66 (1.18, 2.34)	1.26 (0.85, 1.87)
Model 7:	n=1944, 200 events	n=1901, 193 events	n=1925, 200 events	n=1944, 200 events
High	1.00	1.00	1.00	1.00
Moderate	1.36 (0.89, 2.08)	1.32 (0.78, 2.23)	1.44 (0.84, 2.46)	1.22 (0.87, 1.72)
Low	1.32 (0.85, 2.04)	1.53 (0.88, 2.68)	1.69 (1.18, 2.42)	1.25 (0.84, 1.86)

Model 1: univariable analysis including the LTPA component and incident CVD
Model 2: Model 1 + sex, duration of diabetes, age at onset of diabetes and diabetic nephropathy
Model 3: Model 2 + triacylglycerol
Model 4: Model 3 + BMI
Model 5: Model 4 + systolic blood pressure
Model 6: Model 5 + HbA_{1c}
Model 7: Model 6 + history of smoking

LTPA: leisure-time physical activity.

We assessed a small group of individuals with a previous CVD event at baseline (n=106), the association of LTPA and its components with the recurrence-free time of a new CVD event. Of these individuals, 56 had a CVD event during follow-up. Only LTPA intensity was associated with the recurrence-free time (p=0.015). The 10-year cumulative risk of a recurrent CVD event was 68.5% (95% CI, 63.4, 72.9) in the low-intensity group and 45% (95% CI 36.3, 52.5) in the moderate-intensity group, while in the high-intensity group (n=2), there were no events during follow-up. The finding regarding CVD was still statistically significant (HR 1.81 [95% CI 1.04, 3.16]) when adjusted for the static risk factors (gender, diabetic nephropathy, age

at onset of diabetes and duration of diabetes). The association decreased to a non-significant level when the dynamic confounders were added to the Cox regression model (HbA_{1c}, systolic BP, smoking, triacylglycerol and BMI).

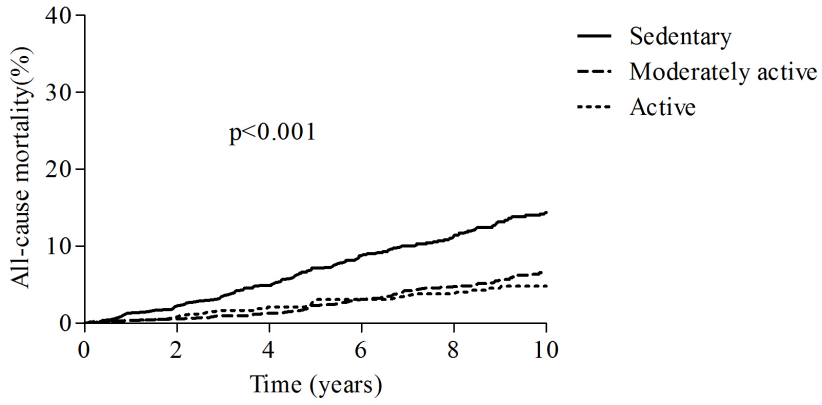
6.3 Study III: The association of LTPA with premature mortality in patients with type 1 diabetes with and without kidney disease

We also assessed how baseline LTPA and its components are associated with all-cause and cardiovascular mortality in individuals with type 1 diabetes in the whole study cohort and separately in individuals with and without CKD.

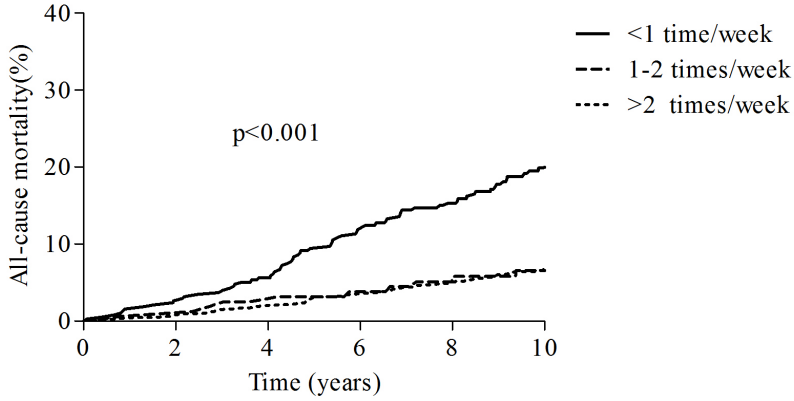
The study population included 2369 participants, and 310 of them had CKD at baseline. During a mean follow-up time of 11.4 ± 3.5 years, 270 participants died from any cause. Of these, 75 (27.8%) were cardiovascular deaths. The baseline clinical characteristics associated with mortality were history of smoking, male gender, higher BMI, age and SBP, longer duration of diabetes, more frequent use of antihypertensive drugs, worse lipid profile and worse glycemic control. The individuals who died were sedentary more often. The baseline risk factors for a cardiovascular death were identical, apart from the history of smoking.

A higher level of LTPA and all its components (intensity, frequency and duration) was associated with the 10-year cumulative rates for all-cause ($p<0.001$) and cardiovascular death (LTPA: $p=0.001$, intensity: $p<0.001$, frequency: $p=0.01$, duration $p=0.02$). The Kaplan–Meier curves for LTPA, its components and all-cause mortality are shown in Figure 6.

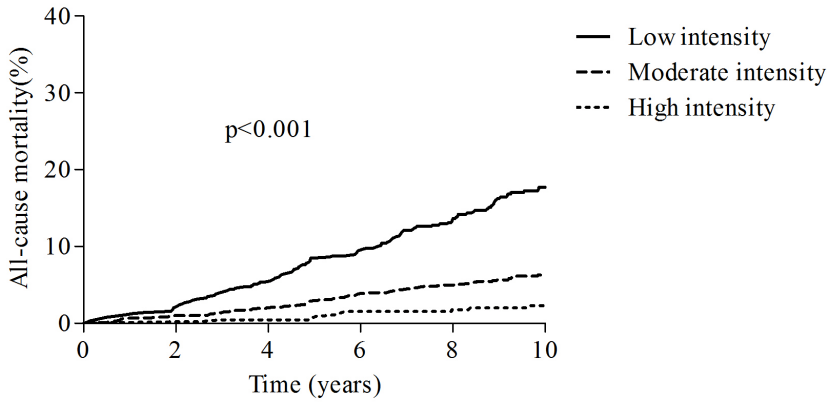
a) LTPA



b) Frequency



c) Intensity



d) Duration

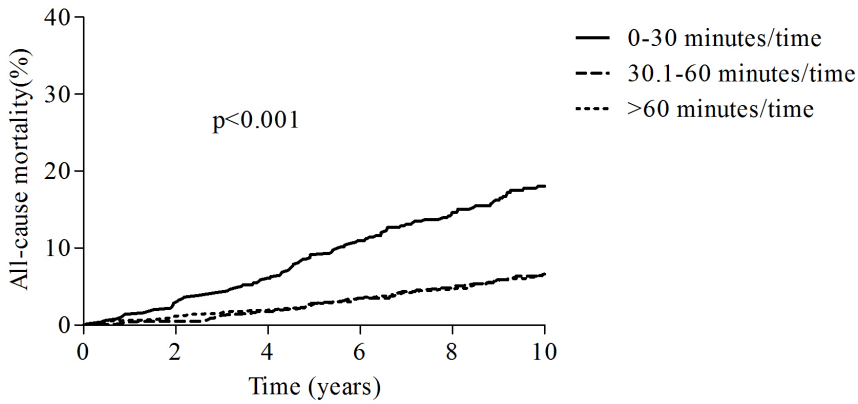


Figure 6. Kaplan–Meier survival analysis for all-cause mortality stratified by total LTPA and its components. LTPA: Leisure-time physical activity.

Next, we assessed the association of all-cause and cardiovascular mortality with LTPA and its components in a layered Cox regression model. The Cox regression models regarding all-cause mortality are shown in Table 6. Model 1 is a univariable model that includes the different LTPA components and all-cause mortality. In model 2, we added information about smoking and the static confounders (gender, diabetic nephropathy, duration of diabetes and age at onset of diabetes). In model 3, we added the dynamic confounders (those that could be affected by exercise): SBP, triglycerides, BMI and HbA_{1c}. Both the total amount of LTPA and all the LTPA components were still associated with all-cause mortality in the final model 3, including both smoking and the static and dynamic confounders. Regarding cardiovascular mortality, the frequency and intensity of LTPA were associated with cardiovascular mortality after adjusting for the static confounders and history of smoking (model 2). However, only the association with cardiovascular mortality and LTPA intensity remained significant after the final adjustment for all potential confounders (model 3).

Table 6. Cox regression models showing hazard ratios for low and moderate vs. high total LTPA, intensity, frequency and duration for all-cause mortality

	LTPA	Intensity	Frequency	Duration
Model 1: High Moderate Low	n=2369, 270 events 1.00 1.11 (0.75–1.64) 2.49 (1.71–3.62)	n=2314, 257 events 1.00 2.55 (1.48–4.39) 7.83 (4.60–13.33)	n=2346, 264 events 1.00 1.13 (0.68–1.89) 2.92 (2.25–3.79)	n=2175, 224 events 1.00 1.08 (0.79–1.48) 2.50 (1.75–3.57)
Model 2: High Moderate Low	n=2315, 261 events 1.00 1.34 (0.89–2.02) 2.07 (1.40–3.06)	n=2261, 249 events 1.00 1.42 (0.80–2.50) 2.78 (1.57–4.90)	n=2292, 255 events 1.00 1.45 (0.86–2.43) 2.35 (1.79–3.09)	n=2126, 217 events 1.00 1.01 (0.73–1.39) 1.86 (1.29–2.68)
Model 3: High Moderate Low	n=2274, 255 events 1.00 1.37 (0.91–2.07) 1.92 (1.29–2.86)	n=2221, 244 events 1.00 1.34 (0.76–2.38) 2.39 (1.34–4.25)	n=2251, 249 events 1.00 1.33 (0.79–2.24) 2.03 (1.53–2.70)	n=2092, 214 events 1.00 1.09 (0.78–1.51) 1.79 (1.23–2.58)

Model 1: univariable analysis including the LTPA component and all-cause mortality
Model 2: Model 1 + gender, duration of diabetes, smoking, age at onset of diabetes and diabetic nephropathy
Model 3: Model 2 + systolic blood pressure, triacylglycerol, BMI and HbA_{1c}

LTPA: leisure-time physical activity.

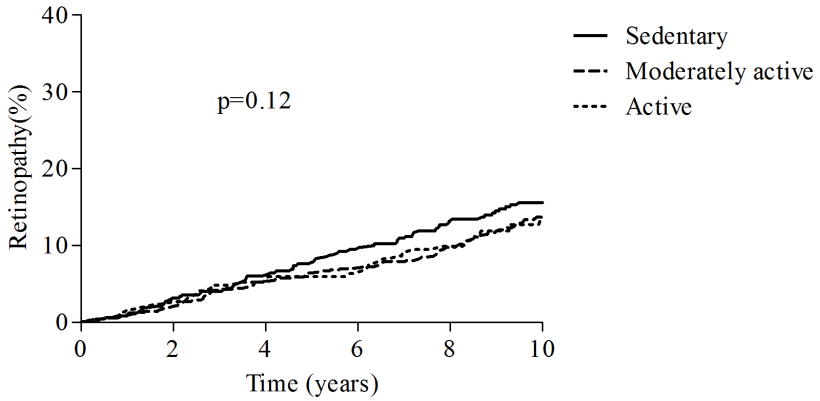
Of the participants with CKD (n=310), 64 had received a kidney transplant, and 36 were undergoing dialysis. During follow-up, 127 deaths occurred from any cause. Both the total amount of LTPA and its components were associated with all-cause mortality in the univariable model 1, and this association remained significant in model 2 when adjusting for the static risk factors and history of smoking. However, only the total amount of LTPA (HR 1.47, 95% CI 1.02–2.12) and LTPA frequency (HR 1.90, 95% CI 1.26–2.87) were independently associated with all-cause mortality in the final model 3, including the dynamic confounders. The findings in the same Cox regression analyses in the participants without CKD were similar to the whole study cohort of individuals with type 1 diabetes: the total amount of LTPA and all the LTPA components were associated with all-cause mortality when adjusting for all the previous confounders (model 3).

6.4 Study IV: The association of LTPA with the development of severe diabetic retinopathy in type 1 diabetes

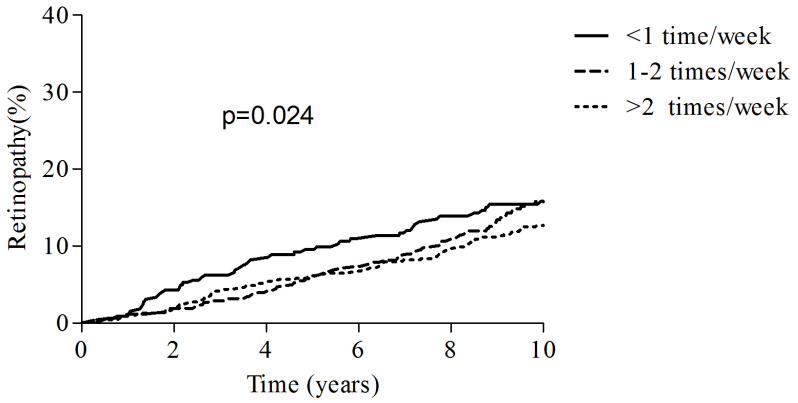
Of the 1612 individuals with type 1 diabetes, 261 developed severe diabetic retinopathy during a mean follow-up time of 10.7 ± 4.6 years. At baseline, those that developed severe retinopathy were younger, had higher blood pressure and BMI, worse lipid profile, higher HbA_{1c}, more often a history of smoking and more frequent use of antihypertensive drugs.

Only a higher frequency of LTPA was associated with a lower 10-year cumulative incidence rate of severe diabetic retinopathy during follow-up, as shown in the Kaplan–Meier curves in Figure 7 (p=0.024).

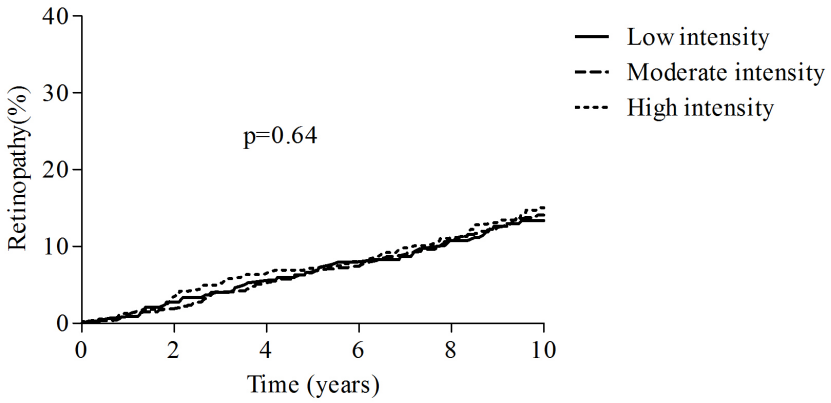
a) LTPA



b) Frequency



c) Intensity



d) Duration

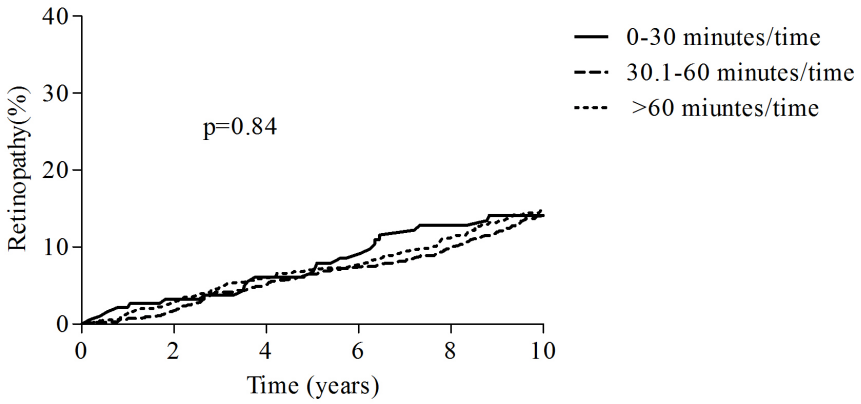


Figure 7. Kaplan–Meier survival analysis for the development of severe diabetic retinopathy stratified by total LTPA and components of LTPA. LTPA: Leisure-time physical activity.

The finding remained significant after adjusting for gender, duration and age at onset of diabetes, kidney function (eGFR), BMI, triglycerides and SBP (p=0.045). When HbA_{1c} and smoking were added to the Cox regression model, the association decreased to a non-significant level. The total amount of LTPA or its components (intensity or the duration of a single session) were not associated with severe diabetic retinopathy. The Cox regression models regarding the total amount of LTPA and all the components are shown in Table 7.

Table 7. Cox regression models showing hazard ratios regarding severe diabetic retinopathy for low and moderate vs. high total LTPA, intensity, frequency and duration

	LTPA	Intensity	Frequency	Duration
Model 1:	n=1612	n=1576	n=1595	n=1498
High	1.00	1.00	1.00	1.00
Moderate	1.10 (0.78–1.55)	0.92 (0.69–1.23)	1.24 (0.93–1.66)	0.92 (0.70–1.21)
Low	1.31 (0.91–1.89)	0.92 (0.64–1.33)	1.40 (1.03–1.89)	0.94 (0.62–1.43)
Model 2:	n=1609	n=1573	n=1592	n=1495
High	1.00	1.00	1.00	1.00
Moderate	1.12 (0.80–1.59)	1.02 (0.75–1.38)	1.21 (0.90–1.62)	0.98 (0.74–1.30)
Low	1.32 (0.91–1.90)	1.01 (0.69–1.47)	1.41 (1.04–1.92)	0.97 (0.64–1.48)
Model 3:	n=1565	n=1529	n=1548	n=1452
High	1.00	1.00	1.00	1.00
Moderate	1.34 (0.94–1.91)	1.03 (0.76–1.41)	1.21 (0.90–1.63)	1.12 (0.83–1.47)
Low	1.27 (0.88–1.85)	0.93 (0.63–1.38)	1.25 (0.91–1.71)	1.00 (0.65–1.54)
Model 4:	n=1518	n=1482	n=1502	n=1408
High	1.00	1.00	1.00	1.00
Moderate	1.32 (0.92–1.88)	0.98 (0.72–1.34)	1.21 (0.90–1.63)	1.08 (0.81–1.44)
Low	1.22 (0.83–1.77)	0.82 (0.56–1.23)	1.18 (0.85–1.62)	0.96 (0.62–1.48)

Model 1: univariable analysis including the LTPA component and severe diabetic retinopathy
Model 2: Model 1 + gender, duration of diabetes, age at onset of diabetes and kidney function
Model 3: Model 2 + systolic blood pressure, triacylglycerol, BMI and HbA_{1c}
Model 4: Model 3 + history of smoking

LTPA: leisure-time physical activity.

7 DISCUSSION

7.1 LTPA and the development and progression of diabetic nephropathy in type 1 diabetes

In these prospective studies, a higher intensity and frequency of LTPA were associated with a slower progression of diabetic nephropathy. To our knowledge, there have been no other prospective epidemiological data assessing the association of physical activity and the development of diabetic nephropathy in individuals with type 1 diabetes. Also, regarding type 2 diabetes, there is no evidence that physical activity affects the risk of the development of diabetic kidney disease.

An alternative explanation for our findings could be that the presence of pre-existing diabetic nephropathy or other diabetic complications would impair the ability to be physically active. For example, exercise intolerance is more common in diabetes, and this might contribute to poor exercise adherence (311, 312). The presence of macrovascular disease and decreased cardiac performance are evident limitations to performing exercise (313). Muscle oxygen supply and utilisation may be impaired due to vascular dysfunction and peripheral ischaemia (314). In addition, autonomic neuropathy is associated with exercise intolerance possibly by causing abnormal haemodynamic responses (315), and diabetic foot ulcers may limit the possibility to perform specific types of exercise. The presence of diabetic nephropathy itself is associated with reduced physical activity levels, decreased physical performance and diabetic complications (316–318).

To partly address this issue of reverse causality with pre-existing baseline renal disease and inactivity, we separately analysed the development of *de novo* microalbuminuria. In these individuals, a lower intensity of LTPA was associated with a more rapid initial progression rate during the 8-year follow-up. However, the differences regarding the progression from normo- to microalbuminuria flattened out during the further follow-up period. Importantly, we demonstrated in another study that our FinnDiane individuals with type 1 diabetes and normoalbuminuria otherwise seem to be of good health despite their diabetes because their mortality was not different from that of the age- and gender-matched non-diabetic Finnish population, which would indicate a low occurrence, for instance, of undiagnosed cardiovascular morbidity, which would reduce exercise tolerance (6).

Our finding regarding the potential importance of physical activity intensity is interesting, and with this finding, we extend our previous cross-sectional results (300). Continuous moderate aerobic exercise has established health benefits and is prescribed in most exercise programs. Interestingly, a number of findings during

recent years show that intensive exercise might provide additional health benefits and may involve distinct mechanisms other than just higher energy consumption. High-intensity interval training has shown greater benefits for improving endothelial function, markers of sympathetic activity, arterial stiffness, insulin sensitivity, blood glucose and lipids compared to continuous moderate-intensity exercise training (319, 320). For example, just three minutes of intensive exercise per week improved insulin action (321), and intensive training of short duration improved glycaemia in individuals with type 2 diabetes (322). Life expectancy was longer with vigorous than with non-vigorous exercise (323, 324). The intensity of LTPA was more important than the total energy output in the association of hypertension and premature mortality (325). LTPA intensity was also associated with reduced risk of CHD (326), as well as reduced levels of subcutaneous fat and WHR (327), independent of the total energy expenditure of LTPA.

The mechanisms of how LTPA may prevent the development of diabetic complications might involve beneficial effects on BP, endothelial function, inflammation, sympathetic load, lipid levels, obesity, glycaemic control and variability and insulin sensitivity. In line with this view, in our multivariable analyses, HbA_{1c}, BP and BMI eliminated the association between LTPA and the progression of renal disease, indicating that these confounders might act at least partially as intermediate variables in the potential protective pathway of LTPA and renal disease.

7.2 LTPA and the risk of premature mortality in individuals with CKD and type 1 diabetes

In Study III, we separately analysed the association of LTPA and premature death in individuals with CKD. Our data show that physical activity is associated with a lower risk of premature mortality in individuals with type 1 diabetes and CKD. All LTPA components were associated with premature mortality when adjusted for the static confounders. However, in the final Cox regression model, including both the static and dynamic confounders, only the total amount of LTPA and frequency of LTPA were associated with premature mortality in these individuals.

To our knowledge, the findings regarding premature mortality are novel. So far, the impact of physical activity on mortality, cardiovascular outcomes or hospitalisation in individuals with CKD is unknown. Previous data show that individuals with CKD are physically inactive and have a lower fitness level and impaired muscle function (316, 328). It appears that physical activity benefits the individuals with CKD: a number of studies show that physical activity is associated with improvements in cardiovascular risk factors in this group of individuals (316, 328). Importantly, physical activity appears to be safe (328). Higher physical activity levels were associated with slower decline in kidney function in those

with CKD during follow-up (329), and no studies have reported a worsening of the kidney function as a result of exercise. Daily 30-minute bouts of moderate-intensity exercise are therefore recommended (316). Our findings are in line with the current recommendations for physical activity in individuals with CKD. Also, these observations were not unexpected given our findings in Study I.

7.3 LTPA and the incidence of CVD events and the risk of premature all-cause and cardiovascular death in type 1 diabetes

In these studies, we showed in a prospective setting that higher LTPA, in particular its frequency and intensity, is associated with a decreased risk of incident CVD events in individuals with type 1 diabetes. In addition, the intensity of the LTPA was associated with recurrent CVD events when adjusted for the static confounders. The intensity of LTPA was associated with cardiovascular mortality when adjusted for many potential confounders. All the LTPA components were associated with all-cause mortality, even in the final multivariable model.

Our findings regarding the association of LTPA intensity with recurrent CVD events and CVD mortality are in line and confirm our previous findings: LTPA intensity was associated with the progression of diabetic nephropathy, and lower intensity was associated with prevalent CVD in a cross-sectional setting. Interestingly, exercise frequency (>2 sessions/week) most strongly associated with the risk of incident CVD. These findings are in line with the current ADA recommendations for diabetes, which include moderate to vigorous aerobic exercise for a minimum of 150 min/week (spreading the activity over at least 3 days weekly) or for 30 min at least 5 days a week. The ADA also recommends going no more than 2 days without exercise. (268)

Our observations are in line with and extend the previous limited data on physical activity, CVD events and mortality. In the Pittsburgh Insulin-Dependent Diabetes Mellitus Morbidity and Mortality study, an inverse association between physical activity and mortality and a weak inverse association between participation in sports during high school and prevalent CVD was observed, but only in men (330). Recently, the EURODIAB study reported a marginally inverse association between physical activity and all-cause mortality in both sexes and a borderline inverse association between physical activity and incident CVD in women only (302). In these studies, however, only the total amount of physical activity was analysed, which may explain why the beneficial effects of physical activity on CVD and mortality were not found in both sexes or why the association was only of borderline significance. Our results show that LTPA is associated with CVD events and mortality in both genders. We also repeated the multivariable analyses for men

and women separately with respect to LTPA and all the components and all-cause mortality. In the final Cox regression model, both the total amount of LTPA and all the exercise components (frequency, duration and intensity) were associated with all-cause mortality in men. In women, on the other hand, only exercise frequency and duration were associated with all-cause mortality in the final model. However, we believe that gender-specific differences regarding the association of physical activity and mortality are unlikely based on previous studies and these current findings.

Contrary to the Cox regression models in Study I, in which the dynamic confounders decreased the association between physical activity and diabetic nephropathy to a non-significant level, the finding regarding LTPA, its components and mortality was unaffected when adjusted for potential confounders. Also, the association between high LTPA frequency and incident CVD events, as well as between LTPA intensity and CVD mortality, were unaffected by the static and dynamic confounders in the final Cox regression model. There are many potential reasons for this. First, there are potential confounders, such as nutrition and socioeconomic status that were not assessed. Second, this might be due to reverse causality. Third, it is likely that multiple factors beyond the traditional CVD risk factors are involved in the potential causal pathway between the larger amount of physical activity and the decreased risk of CVD events and mortality. Factors such as inflammatory markers, insulin sensitivity, endothelial function and physical performance might be involved, but these were not included in our multivariable analyses.

7.4 LTPA and the development of severe diabetic retinopathy

A higher frequency of LTPA was associated with a lower incidence of severe diabetic retinopathy during follow-up. This finding was unaffected when adjusting for gender, renal function, age at onset and duration of diabetes, BMI, systolic blood pressure or triglycerides. However, after adjustment for HbA_{1c} or history of smoking, the association was no more significant. The total amount or the other components of LTPA (intensity or duration of a single session) were not associated with severe diabetic retinopathy.

Previous data on the subject are scarce, and physical activity has been associated with either a significant increase or a decrease in the risk of retinopathy development or its progression. Importantly, the earlier studies have shown no negative effect of physical activity on the development of severe diabetic retinopathy (299, 301, 331). The WESDR showed that participation in high school team sports was associated with lower incidence of diabetic retinopathy, but only in a subgroup of women diagnosed with diabetes under the age of 14 (303). This association remained significant when adjusting for age and duration of diabetes. However, in men or in other individuals in the WESDR cohort, no associations were found.

Our finding extends these previous results to both genders and suggests that there is an association between LTPA and lower incidence of severe diabetic retinopathy.

In type 1 diabetes, glycaemic control, BP and triglycerides are treatable risk factors for diabetic retinopathy, as shown in randomised controlled trials (153, 332–334). In addition, evidence suggests that obesity might also be a treatable risk factor for diabetic retinopathy (335). Physical activity potentially improves these risk factors. The association with frequent LTPA, however, was unaffected after adjusting for these dynamic confounders, suggesting that a potential beneficial effect may be mediated through other factors. These factors could include, for example, vascular endothelial function, insulin sensitivity and inflammation. In our multivariable analysis, the adjustment for HbA_{1c} decreased the association with frequent physical activity to a non-significant level. This might be due to a beneficial effect of physical activity on glycaemia. Physical activity has been shown to improve insulin sensitivity in type 1 diabetes, although the benefit regarding HbA_{1c} lowering has not been established (11, 276). On the other hand, HbA_{1c} may not be an ideal marker of glycaemic control since it does not reflect the glycaemic variability.

Since diabetic retinopathy and diabetic nephropathy are closely associated, we expected that the intensity of LTPA would have been associated with the development of diabetic retinopathy as well since it was found to be associated with the progression of kidney disease. Also, our previous cross-sectional data showed that the intensity was associated with diabetic retinopathy (300). In this longitudinal data set, the incidence of severe diabetic retinopathy was even higher in the higher intensity groups, although not significant. The current ADA recommendations for diabetes and physical activity state that "vigorous aerobic or resistance exercise; jumping, jarring, head-down activities; and breath holding should be avoided in anyone with severe non-proliferative and unstable proliferative diabetic retinopathy" (268). Our observation suggests that these recommendations are well grounded. Further, our finding regarding the potential benefit of frequent physical activity is in line with the current ADA recommendations for physical activity in individuals with type 1 diabetes. However, our rather crude dichotomous classification of severe diabetic retinopathy (yes/no) and subjective measurement of LTPA may reduce our possibility to demonstrate possible associations with the other components of LTPA and identify potential drawbacks or benefits between intensive physical activity and diabetic retinopathy. For example, the EDTRS scale, as well as the thorough characterisation of the retinal status by optical coherence tomography (OCT), could possibly help identify individuals that might benefit and those that might be at risk of further damage by intensive physical activity.

7.5 Methodology – strengths and limitations

7.5.1 Study design and patients

The study population in this thesis includes a cohort of individuals with type 1 diabetes participating in the ongoing nationwide and multi-centre FinnDiane Study. The FinnDiane Study population is comprehensively characterised by medical history, clinical characteristics and presence of diabetic complications. The participation rate in the FinnDiane Study is high (336), and another strength of this thesis is a long follow-up period. Notably, the FinnDiane Study includes more than 10% of the adult Finns with type 1 diabetes, and although it is not by definition a population-based study, the geographical distribution of the study population closely follows that of the general habituation in Finland. The risk of sample selection bias is reduced by recruiting individuals with type 1 diabetes from all levels of the Finnish healthcare system regardless of pre-existing diabetic complications. The study design is prospective and observational, and each study assessed different clinical outcomes: therefore, the cohorts in the sub-studies differ in sample size. However, all individuals with available relevant data were included.

A main strength is the large size of the study cohort of individuals with type 1 diabetes, suggesting that the findings can be considered plausible, valid and clinically important. Furthermore, the longitudinal observations from this thesis address obvious knowledge gaps and the limited amount of pre-existing data on physical activity and diabetic complications in individuals with type 1 diabetes.

Regarding the hierarchy of evidence, a randomised controlled trial (RCT) – considered the gold standard – would provide the strongest level of evidence when measuring the cause-effect relationship of an exposure and an outcome (337, 338). However, a randomised physical activity intervention study assessing the development of diabetic complications and mortality in individuals with type 1 diabetes would be expensive, time-consuming and a relatively inefficient means to assess these outcomes due to their delayed manifestation and the need for a large study cohort. Therefore, observational studies are clinically important for addressing issues that are challenging or that are not addressable through RCTs (337, 339). However, it is important to carefully assess potential sources of biases while conducting observational research, as the risk is higher than in RCTs. Also, the role of potential biases must be considered when interpreting the results of observational studies. The risk for bias can be reduced by careful study design but can never be completely eliminated (338). On the other hand, RCTs have potential biases of their own, including selection biases (340). In addition, it is impossible to conduct a double-blinded RCT when assessing the effect of a physical activity intervention (337, 338).

Although the FinnDiane Study design aims to reduce selection biases, it could not be totally avoided in our assessment of LTPA. The LTPA questionnaire was introduced in 2000 – not in 1997, when the FinnDiane Study was launched – and not all individuals with type 1 diabetes have answered the questionnaire after its introduction. First, the questionnaire is time-consuming to complete thoroughly, as reflected in the amount of information missing in the last section of the questionnaire (work-related physical activity). Second, it is possible that active individuals have completed the questionnaire and answered it more thoroughly than the sedentary individuals.

Ruling out possible confounding factors is a constant challenge in observational studies (338, 341). The basic principle is that a confounder might either overestimate or suggest an association. On the other hand, it might also mask or underestimate a true association. The magnitude and direction of the bias is governed by the associations that the confounders have with the exposure and outcome. (341) In our study, we did address many, albeit not all, potential confounders by conducting Cox regression analyses. For example, nutrition and socioeconomic status were not evaluated by our study. However, we did use smoking as a confounder, and it has been shown to be strongly correlated with socioeconomic status (342). Additionally, we adjusted for many other potential confounders (blood pressure, BMI, lipids, glycaemic control) that are also risk factors for diabetic complications. On the other hand, studies have shown that these covariates themselves are influenced by physical activity (7). In other words, these risk factors might be intermediate variables and may be part of a potential causal pathway between physical activity and diabetic complications (Figure 8, B). Therefore, it would actually be expected that adjustment for these "dynamic" confounders would at least partially blunt the association of physical activity, mortality and diabetic complications. Indeed, this effect was seen in many of our multivariable analyses. The role of a confounding and an intermediate variable is illustrated in Figure 8. It is of note that a variable might simultaneously act as a confounder and as an intermediate variable (343).

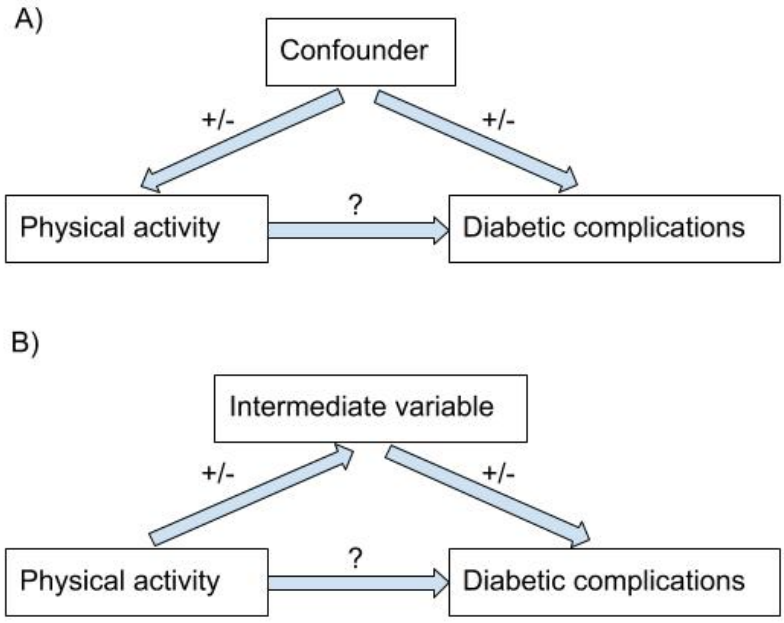


Figure 8. A) A confounder and B) an intermediate variable. Modified from Wakkee et al., "Multivariable Analysis", *Journal of Investigative Dermatology* (2014) and MacKinnon et al., "Equivalence of the Mediation, Confounding and Suppression Effect", *Prevention Science* (2000) (343, 344).

On the other hand, it is not always clear whether the outcome also impacts the exposure, i.e. "the chicken or egg dilemma" (345, 346). Therefore, the role of reverse causality is important to consider when evaluating our observed associations between low levels of physical activity, the development of diabetic complications and premature mortality. Importantly, the presence of diabetic complications at baseline might affect an individual's ability to be physically active. This issue is partly addressed when assessing outcomes in a sub-group of individuals without albuminuria since these individuals are complication-free. However, there is always the possibility that more active individuals or those that perform a higher intensity of physical activity have different beneficial genetic, biological, psychological and social attributes – and the same attributes might also protect this group of individuals from premature death and diabetic complications (346, 347). Irrespective of how important RCTs are for the detection of causalities, the interpretation of the results requires further knowledge derived from observational prospective studies.

7.5.2 Assessment of LTPA

LTPA was assessed using a subjective method. Objective measurement of physical activity is more precise but not applicable to large nationwide cohorts. On the other hand, objective measurements also have their own potential bias such as increased activity during surveillance. In this study, LTPA was self-reported and recalled from the past 12 months, increasing the risk of information bias. Participants might have over- or underestimated their amount and intensity of physical activity, leading to the misclassification of the total LTPA and the variables. Nevertheless, validation studies have shown that, within a cohort, questionnaires are able to classify individuals in rank order according to activity level (348, 349). In addition, the data on work-related physical activity was limited and therefore not assessed, leading to a potential underestimation of the total amount of physical activity. Also, we did not assess the potential changes in LTPA since it was only assessed at baseline. However, based on behavioural studies, it is unlikely that sedentary individuals would become highly active (350). If anything, a possible change in physical activity habits during follow-up might have diluted our results.

Another major strength of this study is that the KIHD LTPA questionnaire has previously been validated in the Finnish population and is custom-made for Finnish conditions. The reliability of the questionnaire has been assessed, and the studies have shown that the questionnaire is representative: the 12-month LTPA correlated with the maximum oxygen uptake with a regression coefficient between the total energy expenditure and the maximal oxygen uptake of 0.23 (351). Further, the questionnaire has been shown to be repeatable and to have a relatively small intraindividual variability (intraclass correlation coefficient 0.58) (308). Another strength is that we assessed not only the total amount of LTPA but also its different components. However, in the present study, the general intensity of physical activity was assessed by a subjective evaluation from the first part of the questionnaire (degree of shortness of breath and sweating). In future studies, however, we should also aim to use a more detailed and validated evaluation of intensity such as the Borg Scale (352).

7.5.3 Assessment of clinical outcomes: the development of diabetic complications and death

So far, the FinnDiane Study has mainly focused on diabetic kidney complications. Therefore, the assessment of kidney status in particular (for example, albuminuria) has been carefully conducted. All medical files and laboratory values have been screened for the assessment of the albuminuria status using the two-out-of-three principles for the classification. Further, registries were used to identify all new initiations of dialysis treatment and transplantations in Finland. Thereafter, these

findings were verified from medical files. If signs of non-diabetic kidney disease were detected at any stage of the assessment, the patient was excluded from the analysis. For the assessment of the other micro- and macrovascular complications, another approach was taken. As a major strength of the thesis, we used nationwide registries (the Care Register for Health Care and the Finnish Causes of Death Registry) to identify these disease outcomes. The registries have been shown to have good coverage and validity (353). Due to the use of these nationwide registries, Studies II–IV lost no individuals to follow-up. Data on mortality were obtained and verified from the Finnish National Death Registry and the centre databases. All deaths were confirmed with death certificate data. The Care Register for Health Care, which was used to identify the CVD events (Study II), has been found to be appropriate for this purpose (353). Also, the information on severe diabetic retinopathy (laser treatment) was obtained from the hospital discharge registry, resulting in complete follow-up data. However, a review of fundus photographs would have certainly given a more precise assessment of the disease status, and this limitation might have resulted in potential disease misclassification both at baseline and at follow-up. However, if anything, this crude classification may have only diluted the findings in Study IV.

7.6 Future directions

In this thesis, we were able to demonstrate several important and novel findings regarding the associations between physical activity, complications and mortality in type 1 diabetes. However, the findings are observational, and therefore, we cannot show direct causality. An ideal study design would be an RCT with hard endpoints; however, as discussed before, this approach involves feasibility issues. Also, the RCTs regarding potential risk factors are scarce, and they have been small and with short duration. Therefore, in the future, it would be important to conduct a well-planned RCT assessing the effect of physical activity on multiple clinical markers and risk factors with the simultaneous control of possible confounders such as nutrition, lifestyle factors and socioeconomic status.

There is a clear need to enlighten the mechanisms of how physical activity benefits the individual with type 1 diabetes and to further establish what benefits the individual the most regarding intensity, duration and type of physical activity. Importantly, the future challenge is to get individuals with type 1 diabetes to be physically active. Physical activity guidelines applied to type 1 diabetes are mostly based on information gained from studies regarding the general population or individuals with type 2 diabetes. Therefore, clinicians need evidence-based instructions on how to prescribe physical activity to these individuals in order to improve the adherence to physical activity programs.

We assessed LTPA with a validated questionnaire, albeit subjectively. In the future, it is important to continue using well-validated tools and possibly to combine subjective and objective tools to assess physical activity. Technology rapidly develops and an intriguing method would be the use of available smart phone applications and data to assess physical activity levels. A combination of this data with a validated questionnaire might enable a more feasible intervention study with a larger study population to also evaluate hard endpoints.

8 SUMMARY AND CONCLUSIONS

I Physical activity, particularly the intensity of LTPA, was associated with the development and progression of diabetic nephropathy in type 1 diabetes during a 6-year follow-up. This was significant after adjusting for "static" confounders such as duration of diabetes, gender and history of smoking. In contrast, "dynamic" confounding factors (HbA_{1c}, BP, triacylglycerol and BMI), which could potentially be influenced by LTPA, incrementally decreased this association to a non-significant level. Also, the frequency of LTPA was associated with renal progression. This association decreased to a non-significant level after adjustment for either the static or dynamic confounders. The total amount or duration of LTPA was not associated with the progression of diabetic nephropathy. We separately analsyed the development of *de novo* microalbuminuria. LTPA intensity was associated with the development of microalbuminuria in a univariable model.

II We investigated the relationship of LTPA and the development of a first-ever CVD event during a 10-year follow-up. Higher LTPA, particularly its frequency and intensity, was associated with a decreased risk of incident CVD events in individuals with type 1 diabetes. LTPA frequency was also associated with incident CVD events in the final multivariable model after adjusting for many confounders (duration of diabetes, age at onset of diabetes, diabetic nephropathy, gender, triacylglycerol, BMI, SBP, HbA_{1c} and smoking). We also separately analysed the relationship between physical activity and recurrence-free time from the baseline visit in a small group of individuals with a previous CVD event at baseline (n=106). Only the intensity of LTPA was associated with the recurrence-free time after a major CVD event at baseline, even after the adjustment of gender, diabetic nephropathy, age at onset of diabetes and duration of diabetes.

III During an average follow-up of 11 years, LTPA and all its components (intensity, duration and frequency) were associated with all-cause mortality even after adjustment for the potential confounders of gender, diabetic nephropathy, duration of diabetes, age at onset of diabetes, SBP, triglycerides, BMI and HbA_{1c} in a study population of 2639 individuals with type 1 diabetes. The intensity of LTPA was associated with CVD mortality after adjusting for all the previous confounders. We separately analysed the relationship between LTPA and all-cause mortality in individuals with CKD and type 1 diabetes (n=310). Of the individuals with CKD, 41% died during follow-up. A higher total and frequency

of LTPA were independently associated with a lower risk of all-cause mortality when adjusted for the previous covariates.

IV Our prospective findings show that physical activity, particularly a higher frequency of LTPA, was associated with a lower incidence of severe diabetic retinopathy in type 1 diabetes during follow-up. The finding was unaffected when adjusting for the potential confounders of gender, renal function, age at onset and duration of diabetes, BMI, SBP or triglycerides. However, after adjusting for HbA_{1c} or history of smoking, the association was no more significant. The total amount of LTPA or its other components (intensity or the duration of a single session) were not associated with severe diabetic retinopathy during follow-up.

General conclusions: The results of this thesis further establish that physical activity is beneficial for individuals with type 1 diabetes and should be an important part of their treatment regimen. In addition, physical activity also seems to benefit those with diabetic complications – notably diabetic nephropathy – and appears to be safe. Therefore, physical activity should also be advocated to these individuals.

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Finally, I dedicate this work to my family. My husband, best friend, Nicolas, I really enjoy everyday life with you. Thank you for your love, encouragement, and support. I am forever grateful for our lovely children Noa and Vivian.

APPENDIX

Physicians and nurses at health care centres participating in the collection of FinnDiane patients.

FinnDiane Study Centres	Physicians and nurses
Anjalankoski Health Centre	S. Koivula, T. Uggeldahl
Central Finland Central Hospital, Jyväskylä	T. Forslund, A. Halonen, A. Koistinen, P. Koskiaho, M. Laukkanen, J. Saltevo, M. Tiihonen
Central Hospital of Åland Islands, Mariehamn	M. Forsen, H. Granlund, A-C. Jonsson, B. Nyroos
Central Hospital of Kanta-Häme, Hämeenlinna	P. Kinnunen, A. Orvola, T. Salonen, A. Vähänen
Central Hospital of Länsi-Pohja, Kemi	H. Laukkanen, P. Nyländen, A. Sademies
Central Ostrobothnian Hospital District, Kokkola	S. Anderson, B. Asplund, U. Byskata, P. Liedes, M. Kuusela, T. Virkkala
City of Espoo Health Centre	
Espoonlahti	A. Nikkola, E. Ritola
Tapiola	M. Niska, H. Saarinen
Samaria	E. Oukko-Ruponen, T. Virtanen
Viherlaakso	A. Lyytinen
City of Helsinki Health Centre	
Puistola	H. Kari, T. Simonen
Suutarila	A. Kaprio, J. Kärkkäinen, B. Rantaeskola
Töölö	P. Kääriäinen, J. Haaga, A-L. Pietiläinen
City of Hyvinkää Health Centre	S. Klemetti, T. Nyandoto, E. Rontu, S. Satuli-Autere
City of Vantaa Health Centre	
Korso	R. Toivonen, H. Virtanen
Länsimäki	R. Ahonen, M. Ivaska-Suomela, A. Jauhiainen
Martinlaakso	M. Laine, T. Pellonpää, R. Puranen
Myyrmäki	A. Airas, J. Laakso, K. Rautavaara
Rekola	M. Erola, E. Jatkola
Tikkurila	R. Lönnblad, A. Malm, J. Mäkelä, E. Rautamo
Heinola Health Centre	P. Hentunen, J. Lagerstam

FinnDiane Study Centres	Physicians and nurses
Helsinki University Central Hospital, Department of Medicine, Division of Nephrology	A. Ahola, J. Fagerudd, M. Feodoroff, D. Gordin, O. Heikkilä, K. Hietala, M. Korolainen, J. Kytö, S. Lindh, K. Pettersson-Fernholm, M. Rosengård-Bärlund, M. Rönnback, A. Sandelin, A-R. Salonen, L. Salovaara, M. Saraheimo, JR Simonsen, L. Thorn, J. Tuomikangas, T. Vesisenaho, J. Wadèn
Herttoniemi Hospital, Helsinki	V. Sipilä
Hospital of Lounais-Häme, Forssa	T. Kalliomäki, J. Koskelainen, R. Nikkanen, N. Savolainen, H. Sulonen, E. Valtonen
Iisalmi Hospital	E. Toivanen
Jokilaakso Hospital, Jämsä	A. Parta, I. Pirttiniemi
Jorvi Hospital, Helsinki University Central Hospital	S. Aranko, S. Ervasti, R. Kauppinen-Mäkelin, A. Kuusisto, T. Leppälä, K. Nikkilä, L. Pekkonen
Jyväskylä Health Centre, Kyllö	K. Nuorva, M. Tiihonen
Kainuu Central Hospital, Kajaani	S. Jokelainen, P. Kemppainen, A-M. Mankinen, M. Sankari
Kerava Health Centre	H. Stuckey, P. Suominen
Kirkkonummi Health Centre	A. Lappalainen, M. Liimatainen, J. Santaholma
Kivelä Hospital, Helsinki	A. Aimolahti, E. Huovinen
Koskela Hospital, Helsinki	V. Ilkka, M. Lehtimäki
Kotka Heath Centre	E. Pälikkö-Kontinen, A. Vanhanen
Kouvola Health Centre	E. Koskinen, T. Siitonen
Kuopio University Hospital	E. Huttunen, R. Ikäheimo, P. Karhapää, P. Kekäläinen, M. Laakso, T. Lakka, E. Lampainen, L. Moilanen, L. Niskanen, U. Tuovinen, I. Vauhkonen, E. Voutilainen
Kuusamo Health Centre	T. Kääriäinen, E. Isopoussu
Kuusankoski Hospital	E. Kilkki, I. Koskinen, L. Riihelä
Laakso Hospital, Helsinki	T. Meriläinen, P. Poukka, R. Savolainen, N. Uhlenius
Lahti City Hospital	A. Mäkelä, M. Tanner
Lapland Central Hospital, Rovaniemi	L. Hyvärinen, S. Severinkangas, T. Tulokas
Lappeenranta Health Centre	P. Linkola, I. Pulli
Lohja Hospital	T. Granlund, M. Saari, T. Salonen
Loimaa Health Centre	A. Mäkelä, P. Eloranta
Länsi-Uusimaa Hospital, Tammisaari	I-M. Jousmaa, J. Rinne
Malmi Hospital, Helsinki	H. Lanki, S. Moilanen, M. Tilly-Kiesi
Mikkeli Central Hospital	A. Gynther, R. Manninen, P. Nironen, M. Salminen, T. Vänttinen
Mänttä Regional Hospital	I. Pirttiniemi, A-M. Hänninen
North Karelian Hospital, Joensuu	U-M. Henttula, P. Kekäläinen, M. Pietarinen, A. Rissanen, M. Voutilainen
Nurmijärvi Health Centre	A. Burgos, K. Urtamo

FinnDiane Study Centres	Physicians and nurses
Oulankangas Hospital, Oulainen	E. Jokelainen, P-L. Jylkkä, E. Kaarlela, J. Vuolaspuro
Oulu Health Centre	L. Hiltunen, R. Häkkinen, S. Keinänen-Kiukaanniemi
Oulu University Hospital	R. Ikäheimo
Päijät-Häme Central Hospital	H. Haapamäki, A. Helanterä, S. Hämäläinen, V. Ilvesmäki, H. Miettinen
Palokka Health Centre	P. Sopanen, L. Welling
Pieksämäki Hospital	V. Javtsenko, M. Tamminen
Pietarsaari Hospital	M-L. Holmbäck, B. Isomaa, L. Sarelin
Pori City Hospital	P. Ahonen, P. Merensalo, K. Sävelä
Porvoo Hospital	M. Kallio, B. Rask, S. Rämö
Raahe Hospital	A. Holma, M. Honkala, A. Tuomivaara, R. Vainionpää
Rauma Hospital	K. Laine, K. Saarinen, T. Salminen
Riihimäki Hospital	P. Aalto, E. Immonen, L. Juurinen
Salo Hospital	A. Alanko, J. Lapinleimu, P. Rautio, M. Virtanen
Satakunta Central Hospital, Pori	M. Asola, M. Juhola, P. Kunelius, M-L. Lahdenmäki, P. Pääkkönen, M. Rautavirta
Savonlinna Central Hospital	E. Korpi-Hyövälti, T. Latvala, E. Leijala
South Karelia Central Hospital, Lappeenranta	T. Ensala, E. Hussi, R. Härkönen, U. Nyholm, J. Toivanen
Tampere Health Centre	A. Vaden, P. Alarotu, E. Kujansuu, H. Kirkkopelto-Jokinen, M. Helin, S. Gummerus, L. Calonius, T. Niskanen, T. Kaitala, T. Vatanen
Tampere University Hospital	I. Ala-Houhala, T. Kuningas, P. Lampinen, M. Määttä, H. Oksala, T. Oksanen, K. Salonen, H. Tauriainen, S. Tulokas
Tiirismaa Health Centre, Hollola	T. Kivelä, L. Petlin, L. Savolainen
Turku Health Centre	I. Hämäläinen, H. Virtamo, M. Vähätalo
Turku University Central Hospital	K. Breitholz, R. Eskola, K. Metsärinne, U. Pietilä, P. Saarinen, R. Tuominen, S. Äyräpää
Vaajakoski Health Centre	K. Mäkinen, P. Sopanen
Valkeakoski Regional Hospital	S. Ojanen, E. Valtonen, H. Ylönen, M. Rautiainen, T. Immonen
Vammala Regional Hospital	I. Isomäki, R. Kroneld, M. Tapiolinna-Mäkelä
Vaasa Central Hospital	S. Bergkulla, U. Hautamäki, V-A. Myllyniemi, I. Rusk

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